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DIRECT BACTERIOLOGICAL EXPERIMENTATION ON THE LIVING MAMMALIAN FETUS *

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INTRODUCTION

The concept of parasitism as a general biological problem cannot be complete without a consideration of the reactions of embryonic and fetal animals. The newborn is not a *tabula rasa* upon which the principles of immunology may be inscribed. It is relatively mature in form and function and reflects in large measure the resistance or susceptibility characteristic of its species. We do not know whether these characteristics are manifest in the youngest embryos or become gradually apparent during the intricate growth period from inception to birth. If susceptibility in any degree parallels morphological development, we may expect fetal reactions to differ widely from those of postnatal animals.

Fragmentary information on the occurrence of spontaneous infections of the fetus has accumulated and congenital transmission of many of the infectious diseases is known to be possible. Such transmission is only occasional and usually near term. More data are necessary before we can judge whether the younger fetuses are more resistant to infection or merely less exposed thereto.

Most of the investigations dealing with experimental fetal infections have been concerned with passage of the infectious agent from maternal to fetal circulations through the placenta. In experiments of this type the amount of inoculum that actually reaches the fetus and the time of effective fetal inoculation remain unknown. Many factors affect the result, such as the concentration of the organism in

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the maternal blood stream, the species type of placenta, and the degree of placental damage.

In view of these considerations a more direct approach to the problem of parasitism of the fetus appears desirable. But further than this, there is ample justification on a practical basis for this type of experimentation. Much of the work of the bacteriologist is dependent on the use of experimental animals in the study of both bacteria and viruses. Yet we know that there are definite and characteristic limitations in the use of any particular animal for any given parasite. It is important to determine whether some of these limitations might be modified by extending the host range to include the fetus. Furthermore, the fetus occupies a unique situation, being in an environment of constant temperature and nutrition and well protected from chance contamination and external influence. It constitutes what might be termed a "test tube animal." Such conditions appear ideal for the facilitation of certain types of bacteriological studies.

Technique

Isolated reports of fetal experimentation may be found in the literature published by Charrin,[1] Kreidl and Mandl,[2] Wolff,[3] Bourquin,[4] Bors,[5] Nicholas,[6] Debrunner,[7] Wohlwill and Bock [8] (1933), and others. These studies have been concerned chiefly with pathological changes or with the passage of various substances through the placenta. The work of Bors particularly established the practicability of experimentation on mammalian fetuses.

It is apparent that the purposes of the bacteriologist will not usually require such an elaborate technique as might be demanded for delicate intrauterine surgery in anatomical or similar studies. In the work reported herein we have used the most simple technique consistent with well controlled bacteriological procedure.

Guinea pigs, rabbits and a few rats were used. Most of the work was done on the guinea pig and the remarks herein will refer to that animal unless otherwise indicated. In general, we found the guinea pig the most adaptable to this type of experimentation. They care for their young better, have a longer period of gestation, and tolerate repeated surgical procedures well. The stock animals were kept in large breeding pens and suitable pregnant animals selected from time to time. With a little experience one can judge the approxi-

mate fetal age by palpation. This is possible from about the 20th day of gestation until term at about the 65th day.

Several different methods of inoculation were used, depending upon the fetal age. Until about the 30th day the fetus is so small in relation to decidua, placenta and amniotic fluid that direct inoculation is impracticable. For this stage intraplacental inoculation was employed. The mothers were anesthetized either by drop ether from a separatory funnel, by sodium amytal (Lilly) given hypodermically in a dosage of about 50 mg. per kilo body weight, or by a smaller dose of sodium amytal followed by ether. Following midline laparotomy under aseptic precautions, the uterus was delivered, the number and location of fetuses in the two uterine horns determined, and the placentas inoculated by needle puncture through the uterine wall. The abdominal wall was closed in one layer, the skin with heavy interrupted sutures, and collodion was applied to the incision. The placental method of inoculation is necessarily open to the objection that there can be no control of the distribution of inoculum between maternal and fetal blood sinuses in the placenta.

Fetuses from about 30 to 40 days of age were inoculated by needle puncture through the maternal uterine wall and fetal membranes, following surgical exposure of the uterus as described above. At this age the fetuses are readily palpable, the fetal head particularly being easily identified and inoculated. Most of our inoculations were made at this period of gestation and in this way. Needle puncture through the uterine wall is associated with a certain amount of contamination of maternal tissue with inoculum intended for the fetus. In most of our experiments such maternal contamination was not considered important. To avoid this, in certain instances the fetus was exposed by a small incision through the uterine wall and fetal membranes. Following direct needle inoculation the site was cauterized to destroy surface traces of the inoculum.

Older fetuses are more likely to be aborted if the mother is subjected to the full operative procedure. In some cases such fetuses were inoculated through small incisions in the maternal abdominal wall immediately over the fetal part desired. We found it possible also to inoculate older fetuses intracerebrally by needle puncture through the intact maternal abdominal wall. The fetal calvarium becomes calcified long before term and in the later fetal stages can be identified and manipulated against the maternal abdominal wall,

particularly if the mother is well relaxed with anesthesia. Although the danger of perforating some interposed viscus appears great, in no instance was there any evidence that such a thing occurred, nor did any of these mothers abort.

The termination of our experiments was principally by cesarean section. With such a procedure the several fetuses could be identified by their respective positions in the uterus. If the mother is allowed to go to term such identification is not possible and there is the danger that some of the fetuses born dead or alive may be destroyed by the mother. In several instances we have performed serial cesarean sections a week or more apart on the same litter. Occasionally we have delivered mature fetuses by section in order to identify them and then permitted them to be reared by foster mothers. Usually the mothers were sacrificed at section but in many cases the operations were completed surgically, the mothers were re-bred and used again, some of them repeatedly, in similar experimentation.

Experimental

The following antigens were selected for inoculation in fetal guinea pigs:

(1) The virus of poliomyelitis, for which the monkey is at present the only susceptible experimental animal.
(2) The vaccinia virus, which finds a relatively resistant host in the guinea pig.
(3) The submaxillary gland virus of guinea pigs, a virus natural to that animal and latent in spontaneous infections.
(4) The tubercle bacillus (H37) and Bacille Calmette-Guérin (B C G), representing virulent and relatively non-virulent strains, respectively.
(5) Diphtheria toxin, for the study of which the guinea pig may be said to be the classical experimental animal.

(1) *Poliomyelitis Virus*

A chemically purified and concentrated virus of known infectivity for monkeys was passed through two series of fetal guinea pigs, one at 5 day, the other at 10 day intervals. Eight transfers were made in the former series and four in the latter. The fetuses were 30 to 50 days old. All inoculations were made intracerebrally by needle

puncture through the uterine wall. Fetuses to be used as a source of subinoculation material were removed through cesarean section. From each fetus of the litter one lateral half of the brain was removed aseptically for subinoculation; the other half was preserved for histological examination. The passage material was prepared by pooling the brain tissue from the several fetuses of the litter, grinding with sand in a sterile test tube and suspending in saline solution.

In none of the fetuses were there gross or histological changes that could be interpreted as representing a specific effect of the virus. Material from the sixth subinoculation (5 day series) failed to produce poliomyelitis administered intracerebrally to a monkey. Frequent bacteriological cultures of fetal brain and heart blood in beef heart broth and on blood agar plates were consistently negative.

Comment: The above experiments are of course not extensive enough to warrant final conclusions as to the susceptibility of this fetal animal to poliomyelitis virus. Inoculations by other routes, at other ages, through larger series, and in other species would be necessary for complete exploration of the problem. From work reported by Stritar and Hudson [9] on the vaccinia virus, it is evident that the concentration of a virus can vary greatly in the different fetal organs. Perhaps the poliomyelitis virus would find a more favorable medium for growth in some fetal organ other than the brain. Within the limitations of the experiment, however, it is clear that the fetal guinea pig manifested the resistance characteristic of its species to poliomyelitis virus infection. The fact that the fetal tissues remained bacteriologically sterile throughout the course of transfer is noteworthy in view of claims of a bacterial form of the virus.

(2) *Vaccinia Virus*

A neurotropic strain (Levaditi), maintained in tissue culture, was employed. Its dermal titer in rabbits was 1: 10,000. When injected intracutaneously in adult guinea pigs in 1: 10 dilution it caused only a faint local reaction and no febrile response. Administered intracerebrally to fetal guinea pigs in 1: 100 dilution it was found to disseminate widely and often produced death within 5 to 7 days. The sites of predilection for gross lesions seemed to be skin and lungs, although lesions were occasionally seen in liver, brain and elsewhere. The cutaneous lesions stood out sharply on the hairless fetal skin as

pale, slightly raised nodules from 0.5 to 3 mm. in diameter, some of which progressed to ulceration. The virus could be recovered in high titer from these lesions and its specificity demonstrated by rabbit inoculation.

Comment: This is an example of a fetal animal that appears to be much more susceptible than the newborn or adult to a particular virus infection. Although guinea pigs have at times been used extensively in ophthalmic tests for vaccinia virus, and Bland [10] has shown that the virus may be adapted to the guinea pig by serial transfer to the degree that it causes skin lesions, there is a general and well founded belief that the guinea pig is relatively resistant to vaccinia infection.

(3) *Submaxillary Gland Virus of Guinea Pigs*

Four different preparations of fresh guinea pig submaxillary gland material were injected into fetal and newborn guinea pigs concurrently.* The results may be summarized by stating that for all four preparations there was a positive correlation between the effects in fetal and newborn animals. One of the preparations was innocuous in both. The other three were effective in dilutions up to 1:1000 in the fetus. Widespread and characteristic lesions and sometimes death resulted. The effects on individual fetuses of a litter were proportionate to the amount of the material injected and the duration of the experiment.

Comment: In this instance we are exposing the fetal guinea pig to a virus native to the species yet usually latent in spontaneous infections. Most adult guinea pigs may be considered to possess an "infection immunity," since they manifest no active symptoms of the disease and still carry the virus in an infectious state. Interesting problems in immunology are thus raised. One might expect that immune adults would passively protect their young. However, Markham and Hudson [11] have observed that fetuses can be definitely infected even though borne by spontaneously infected mothers. One may infer, therefore, either that the maternal immunity is not humoral or that antibodies do not pass the placenta in effective quantity.

* This material was obtained from Dr. F. S. Markham and the inoculations of newborn guinea pigs referred to were made by him (Markham and Hudson [11]).

In these experiments and others the practicability of titration of infectious material in a single litter has been seen. In no case has there been any evidence of transfer of the infectious agent from one fetus to another within the same litter. Control fetuses have never been involved.

(4) *Bacillus Tuberculosis and Bacille Calmette-Guérin*

The H_{37} virulent human strain of *B. tuberculosis*, grown on Long's synthetic medium, was prepared in weighed amounts for inoculation as a suspension in saline. The guinea pig fetuses were injected intracerebrally, for the most part. All such fetuses died within a short time, some *in utero* and others soon after birth. Characteristic changes, both local and metastatic, were evident. Meningitis and necrosis of the fetal brains were often very marked. The liver and spleen usually contained tubercles if the infection had persisted for more than a few days.

The B C G was prepared and injected in like manner. Again, changes characteristic of tuberculosis resulted. Tendency to distribution, however, was much less, and the local lesions were less severe than those produced by comparable dosages of the virulent tubercle bacillus.

Comment: Since the guinea pig is a classical animal for tuberculosis work, the susceptibility of the fetal guinea pig is not surprising. Whether the fetus is relatively more susceptible than newborn or adult animals was not determined in these preliminary experiments. They are presented here to illustrate possible advantages of using the fetus in virulence tests. Such experiments have always been open to the objection that the animal may become accidentally infected with extraneous organisms. The fetus, however, is not subject to chance infection, so that the effects of direct inoculation or of serial passage must be given full credence (Neiman and Woolpert [12]).

A point of interest in fetal experiments on tuberculosis was that pathological changes often proceeded *in utero* far beyond the stage that would have been compatible with life in the external environment. The brains of some of these fetuses were almost entirely destroyed, yet the animals lived and developed normally, succumbing promptly, however, when delivered into the outer world as autonomous beings.

(5) *Diphtheria Toxin*

A ripened, standardized toxin, obtained through the courtesy of Parke, Davis & Co., was titrated in 6 newborn guinea pigs and 8 fetuses in three litters. The actual amounts of toxin injected into the fetuses ranged from 0.1 cc. of 1:1280 dilution to 0.1 cc. of 1:20 dilution. All of the fetuses were delivered by section from 2 to 9 days following inoculation. Protocols for the fetal inoculations are set forth in Table I. Table II gives a summary of the results

TABLE I

Diphtheria Toxin Titration in Fetal Guinea Pigs

Guinea pig No.	Approximate days of pregnancy	Day experiment terminated	Fetus No.	Approximate weight of fetus	Inoculum		Results
					cc.	Dilution of toxin	
				gm.			
291	40	49	1	10	0.10	1:1280	Living; weight 31.2 gm., grossly normal
			2	10	0.10	1:640	Living; weight 31.6 gm., grossly normal
290	35	37	1	5	0.05	1:320	Living; weight 5.5 gm., grossly normal
			2	5	0.05	1:160	Living; weight 6.8 gm., grossly normal
			3	5	0.05	1:80	Dead; weight 6 gm., marked postmortem change
295	38	40	1	10	0.10	1:80	Dead; weight 10 gm., early postmortem change
			2	10	0.10	1:40	Dead; weight 12.5 gm., early postmortem change
			3	10	0.10	1:20	Dead; weight 10.3 gm., extensive postmortem change

computed on a basis of cubic centimeters of toxin per kilogram body-weight of animal and shows that the minimal lethal dose for both newborn and fetus was between 0.05 and 0.10 cc.

Comment: These experiments imply that the fetal guinea pig is as reactive as the newborn to the action of this toxin; in fact there is a surprising agreement of titer, considering the small number of animals used. It was shown by Schmidlechner [13] in 1904 that fetal guinea pigs could be killed *in utero* if sufficient toxin were injected into the mother to provide an excess over the amount that would be bound by the maternal tissue. Nevertheless, the impression has

been general that young animals are relatively insusceptible to classical toxins because of a lack of "receptors" or for other theoretical reasons. The fetuses used in our experiments were from one-twentieth to one-fifth the weight of newborn guinea pigs, yet they were acutely sensitive to the small amounts of diphtheria toxin injected. Histological studies were not made. Again, the practicability of titrations in fetal litters is suggested.

TABLE II

Comparative Reaction of Newborn and Fetal Guinea Pigs to Diphtheria Toxin

Newborn guinea pigs	Fetal guinea pigs
0.1 cc. per kilo — lethal	0.5 cc. per kilo — lethal
0.05 " " " — sublethal	0.25 " " " — lethal
0.033 " " " — sublethal	0.125 " " " — lethal
	0.0625 " " " — sublethal
	0.0312 " " " — sublethal
	0.0156 " " " — sublethal
	0.0073 " " " — sublethal

DISCUSSION

Our interest in the fetus as an experimental animal was the outgrowth of efforts to find a more convenient animal than the monkey for poliomyelitis studies. Although this hope failed of realization there appeared sufficient promise in the work to warrant extending its scope to include other infectious agents. The major technical problems encountered were: first, that of inoculating the fetuses without jeopardizing the life of the mother, or producing abortion; and second, that of observing the course of events following inoculation and recovering the virus and fetal tissues at the stage desired. In our experience maternal death occurred rarely and abortion did not commonly take place if healthy mothers at the appropriate gestation stage were used. Graded inoculations and a few trial experiments with the particular virus enabled us to judge fairly accurately the proper time for terminating an experiment. It is hoped that the technique can be modified to permit experimentation on both younger and older fetuses and to include other species. The actual experimental results in this report should be considered as preliminary and suggestive rather than final. More extensive and detailed

experiments are reported elsewhere by Neiman and Woolpert,[12] Stritar and Hudson,[9] and Markham and Hudson.[11]

Any generalizations in theory concerning fetal resistance to infection would be unwarranted at this time. *A priori*, one might favor one or the other of two views: either the fetus in its primitive state should constitute merely good culture medium, devoid of cellular or humoral defense; or, on the other hand, it should be resistant because of its very immaturity. So far as histological reaction is concerned, the impression is general that embryos and younger fetuses are relatively non-reactive. Wohlwill and Bock [8] (1930) could not demonstrate inflammatory reaction in the human fetus under 11.5 cm. in length. From this stage on, a localized histiocytic reaction became gradually evident. Granulocytic infiltration was seen only in the more mature fetuses. In experimental studies the same authors [8] (1933) found that fetal guinea pigs were histologically relatively nonreactive to the bacterial and chemical irritants used. However, lack of histological response does not necessarily imply absence of tissue damage or unsuitability for the support of bacterial growth. We know that embryonic tissues have been found particularly favorable for the propagation of many viruses in tissue culture. In our experience with the fetal guinea pig there were marked tissue changes and often death associated with infection by the tubercle bacillus and the submaxillary gland virus of guinea pigs. The histological changes produced by vaccinia virus were less extensive but death often resulted. Although fetuses were killed by small amounts of diphtheria toxin there was probably little characteristic gross change. The poliomyelitis virus exerted neither vital nor cellular effects. It may be that fetal reactions, like those of adults, will be found to be individualized, with respect to both host and parasite.

Summary

A technique for direct bacteriological experimentation on the living fetus of small laboratory mammals is described.

The theoretical and practical advantages of such experimentation are discussed.

The results of preliminary experiments illustrating the applications and limitations of this technique are briefly reported.

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INTRACEREBRAL INOCULATION OF FETAL GUINEA PIGS WITH BACILLE CALMETTE-GUÉRIN AND THE H_{37} STRAIN OF TUBERCLE BACILLUS *

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INTRODUCTION

The administration of Bacille Calmette-Guérin (B C G) to infants represents the most popular method in use today for the purpose of protecting man from tuberculosis. It was thought that the production of a subclinical, spontaneously healing infection in man would at least increase resistance to subsequent infection of natural origin. Nocard in 1906 isolated a strain of bovine tubercle bacillus which Calmette and Guérin grew on 5 per cent glycerine-bile-potato medium. They [1] proclaimed this bacterium to be attenuated, after fifteen transfers on this medium, to the degree that it no longer caused progressive tuberculosis in cattle or guinea pigs. Experiments were begun in the protection of animals and by 1922 results were judged so uniformly successful that Weill-Hallé [2] ventured to give the vaccine to infants.

At present B C G is an organism well known to clinicians and investigators interested in tuberculosis. As a vaccine against this disease it is not universally accepted. The controversy centers about the statement made by Calmette that B C G is a "virus fixé," meaning that it is permanently attenuated and not capable of regaining its former pathogenic properties under any environmental conditions. The evidence for and against the ability of B C G to change in virulence by growth on artificial media is not balanced. By far the greater number of workers claim that the known methods of artificial cultivation do not cause B C G to become virulent. In addition, only partial success has attended attempts to produce progressive tuberculosis with this bacterium in experimental animals, as illustrated by the work of Feldman.[3] Transfer of B C G

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from animal to animal has not been accomplished except when a progressive disease has been induced.

Calmette and his supporters have repeated the experiments in the literature purporting to show that B C G is capable under certain conditions of regaining virulence or of producing progressive tuberculosis. The reports of Birkhaug [4] and of Boquet [5] deal with these repetitions and show only negative results. In explanation they affirm that the smooth variant of Petroff, Branch and Steenken [6] and the tuberculosis allegedly caused by B C G are really due to a contamination of the inoculum or to an intercurrent infection of spontaneous origin. In order to eliminate these criticisms, one should use experimental animals known to be tuberculosis-free and maintained in that condition.

The feasibility of using the fetus as an experimental animal has been shown by Woolpert.[7] He has developed a technique whereby fetuses may be inoculated *in utero* without interruption of the gestation period. Such a technique opens many avenues to research and is particularly applicable to the problem of B C G, since the fetus is protected from exposure to intercurrent infection. At most, the remote possibility of transplacental tuberculous infection obtains only when the mother has an overwhelming infection. Another possible advantage of employing this experimental method with B C G is that the fetus may be more susceptible than young or adult animals to relatively avirulent organisms. In this respect also it may constitute a favorable medium for passage experiments in attempts to convert B C G into a more virulent form.

Experiments with the H37 Strain of Tubercle Bacillus

It was thought desirable to determine the reaction of the guinea pig fetus to a standard virulent strain of the tubercle bacillus in order to establish a basis for judging the results with B C G. For this purpose H37 was used. It was originally isolated by Baldwin in 1905 from a case of human tuberculosis and has since been subcultured on artificial media. In this laboratory it has been maintained on Long's synthetic medium.

Accurately determined amounts of tubercle bacilli calculated on the basis of dry weight were injected intracerebrally into fetuses. The age of the culture varied from 25 to 55 days. In inoculating

fetuses by this route it was necessary to pierce the uterine wall and the fetal membranes and calvarium. It is probable that some of the inoculum entered the maternal tissue but gross tuberculosis was rarely observed, being seen in only 1 of 15 mothers whose fetuses were injected with H37. There was no evidence that maternal infection influenced the fetal experimentation.

Thirty-six fetuses in 15 mothers were inoculated with H37. Of these, 13 were alive and 11 were dead when removed by cesarean section; 6 were delivered dead and 2 died several days after delivery; and 4 survived the inoculation of heat-killed bacilli. The dosage varied from 0.01 mg. to 1 mg. The fetuses were removed by cesarean section 5 to 28 days after injection and were examined for signs of life. An autopsy was performed and the tissues were inspected grossly and microscopically. The brains were cultured for tubercle bacilli on Herrold's egg medium and smears of the brains were stained by the Ziehl-Neelsen method for acid-fast bacteria.

The local reaction most commonly observed was a meningitis which was infrequently grossly visible. The extent to which the meninges were involved varied directly with the dosage and with the interval between inoculation and examination. Caseous nodules were discernible with the naked eye in the basal meninges in those fetuses delivered dead and in those that died soon after birth (Fig. 1).

In the brains of most fetuses, except those that had received 0.01 mg. and were examined in the early stages, an internal hydrocephalus was present. Grossly it was impossible to determine where the obstruction to the flow of cerebrospinal fluid was taking place, but many microscopic sections disclosed tubercles at the base of the brain, extending into and occluding the foramina of Luschka (Fig. 1).

The dissemination of the tubercle bacilli to other organs was followed histologically in sections stained by the Cooper modification of the Ziehl-Neelsen technique.[8] About 3 weeks after inoculation with all dosages, gross tubercle formation was noted in the liver, spleen and lungs. Microscopically it was found that the liver was the first organ to be affected. Bacilli appeared here as early as 5 days after intracerebral inoculation even with the smallest dose. The next organ to be involved was the spleen, in which the appearance of bacilli was variable. Sometimes they appeared early in the course of the infection and at other times late. Some fetuses inoculated with as much as 1 mg. of H37 did not show the bacilli in the spleen at

the end of 2 weeks. The lungs were found infected after a period of 3 weeks in fetuses receiving 0.01 mg. and in 1 week in those inoculated with 0.1 mg. Infrequently the placenta was found to have miliary tubercles. The age of the fetus did not have any definite relation to the experimental outcome, all showing dissemination of H37 at least to the liver. Table I summarizes these findings.

Fetuses Dead in Utero and After Delivery: There were 11 of the former class removed by cesarean section at intervals varying from 5 to 22 days. Ten were in a state of mummification and 1 was calcified. Tissue smears showed acid-fast bacilli. Six animals dead immediately after birth had generalized tuberculosis. Acid-fast bacteria were demonstrable in representative tissues both in histological sections and in stained smears. Two animals that died 11 and 19 days after delivery also showed a generalized tuberculosis.

Controls: Four fetuses were inoculated with H37 killed by heat (60° C. for 1 hour). Two of them that had received 0.1 and 1 mg. of the dead bacteria were removed by cesarean section 6 days after inoculation. No gross tuberculosis was found and microscopically there was only a localized meningitis in which intracellular and disintegrating bacilli were present. The other 2, inoculated with 0.1 and 0.01 mg., were delivered and allowed to live for 15 months, when they were sacrificed; autopsy showed no tuberculosis.

Recovery of the tubercle bacilli on an artificial medium (Herrold's egg) from fetal brains was effected in ten instances out of twelve attempts. The organisms did not seem to have changed from the originally inoculated bacteria in microscopic appearance, staining reaction or colony characteristics.

Inoculation of Newborn: Seven guinea pigs 4 to 5 days old were inoculated intracerebrally with H37. One received 0.01 mg., another 0.1 mg. and 4 were injected with 1 mg. All of these animals died of generalized tuberculosis in 15 to 28 days after inoculation, depending on the dosage. The gross and histological pathology was very similar to that seen in the fetuses.

The microscopic structure of the tubercles in the fetus depended on their age when examined. It was generally found that the polymorphonuclear leukocytes appeared first and were closely followed by large mononuclear cells with pale staining nuclei. Tubercles in fetal tissues examined early in the course of the disease were composed chiefly of the polymorphonuclears with only a few large mono-

nuclears. In the later stages the tubercles were seen to have caseating centers, invaded and surrounded by the large mononuclear cells. Later still, lymphocytes appeared and encircled the entire area. No giant cells were seen that could be definitely identified as the typical Langhans variety.

The tubercle bacilli themselves were found extracellularly and intracellularly, chiefly in the large mononuclear cells. Occasionally in the early stages of the disease they were lying free in the tissues with practically no reaction about them. They had a granular appearance and were often seen in groups lying parallel to each other. This last, according to the analysis by Woodruff,[9] indicates growth and multiplication (Fig. 5).

In addition to the distention of the ventricles and the meningitis already described, the ventricular wall and the brain tissue proper were invaded by tubercle bacilli which set up caseating foci there. Here, as in many of the other tissues, was visible the phenomenon of satellite tubercles. This is supposed to be indicative of invasiveness of the bacilli since the "satellites" are obviously (from their cell structure) younger than the central tubercle. In the liver the portal triads were most often the site of tubercle formation. There was no predilection for any specific structural unit in the lungs and spleen, although in the latter, reactions frequently occurred in or near malpighian bodies. Tubercles in the placenta were often in the maternal sinuses.

Summary: A series of fetal guinea pigs were inoculated intracerebrally with graded doses of H37 and if not dead were killed at the end of a predetermined length of time and then examined grossly, histologically and bacteriologically. The results indicate that H37 establishes in the guinea pig fetus a progressive tuberculosis in certain respects similar to that produced by this bacillus in the adult animal.

Experiments with B C G

The B C G cultures used in these experiments were from two sources. The one kindly sent us by Dr. William H. Park was used in the greater number of experiments. The other from Paris, through Dr. Florence Seibert, was used only in the early experiments. Cultures were grown in Sauton's liquid and Long's synthetic media, and suspensions of it were prepared in the same manner as described for

H37. The B C G was kept in a separate incubator from that in which the cultures of H37 were grown. Likewise, animals whose fetuses received the former were caged in a different room from those whose fetuses were inoculated with H37.

Inoculations of 1.4 to 3 mg. Amounts of B C G: These were made intracerebrally into fetal guinea pigs of varying ages. They were examined for signs of tuberculosis after certain time intervals. The large doses were used in initial experiments because of the purported avirulence of B C G for adult guinea pigs. Seven fetuses in 3 mothers were inoculated through the maternal abdominal wall with 3 mg. of the bacilli from 5 to 7 days before delivery. All the fetuses died of generalized tuberculosis from 1 to 45 days after delivery. Ten fetuses in 3 mothers were inoculated, after aseptic laparotomy to expose the uterus, with 2 mg. of this bacterium. Four died of generalized tuberculosis immediately after birth. The remaining 6, when examined *in utero* 2 weeks after inoculation, were found to be tuberculous although alive. Three fetuses in 1 mother were injected with 1.5 mg. They were removed by cesarean section 8, 14 and 20 days after inoculation. The tuberculosis was found to be progressively greater in each fetus, the last being dead when removed. Four fetuses in 1 mother were inoculated with 1.4 mg. and died of generalized tuberculosis immediately after delivery, 31 days after inoculation. The diagnosis of tuberculosis in these fetuses and newborn was based on gross and microscopic evidence.

Experiments with Smaller Amounts of B C G: The results of the above experiments were attributed to large doses. Accordingly, a series of 35 fetuses in 16 mothers were inoculated by the same technique described for H37 with doses ranging from 0.01 mg. to 1 mg. Only 5 fetuses were inoculated with 0.01 mg. because this dose was early found to produce very little pathology. Three fetuses receiving this amount were delivered naturally. One was dead at birth but showed no tuberculous changes; 1 was killed and autopsied at 6 months of age and found to be essentially normal; the other is still alive (15 months after inoculation). Two fetuses were examined *in utero* 14 days after inoculation. Both were alive; 1 was apparently normal in every respect, and in the other there were minor histological changes.

Twenty-seven fetuses were inoculated with 0.1 and 1 mg. amounts of B C G. Of these, 2 were killed 2 days after delivery (35 days

after inoculation), 2 died immediately after birth, and 1 died 1 day and another 4 days after delivery. All of them showed generalized tuberculosis at autopsy, manifested by caseous nodules in the brain and liver, and a gray and nodular spleen. A single animal that received 0.1 mg. of the inoculum is still alive 1 year after inoculation.

The 20 remaining fetuses were removed alive by cesarean section from 7 to 28 days after inoculation. The only gross change locally was a distention of the lateral ventricles, which was explained in most instances by a microscopic tubercle occluding the foramen of Luschka. Also, microscopically a meningitis was demonstrable in most of the fetuses. Only with the larger dosages and after the longer intervals were tubercles found in the brain tissue proper.

The distribution of the inoculum to other organs was followed histologically and it was found that the liver was the most frequent site of metastasis. The lungs and spleen were often sites of deposition of the bacilli but the placenta was only infrequently involved. However, dissemination was not as rapid as seen with H37. Those fetuses inoculated with 0.1 mg. did not show tuberculous involvement of organs, aside from the brain, except after the longer intervals, but those receiving 1 mg. manifested a rapid spread. In Table I these findings are summarized and compared to the observations made on H37 in this respect.

Controls: Nine fetuses were inoculated with heat-killed bacilli. All were delivered alive and apparently normal, but 3 died soon after birth. Autopsy showed an internal hydrocephalus and a localized meningitis in which no tubercle bacilli were demonstrable. All the other organs were essentially normal. No tuberculous pathology was found in the other 6 when they were killed and autopsied 6 months after delivery.

The tubercles found in the fetuses receiving B C G had the same cellular structure and showed the phenomenon of satellite tubercles as described for those produced by H37. The acid-fast bacilli had the same microscopic appearance as H37 and were extracellular as well as intracellular (Figs. 4, 5 and 6). They were in the large mononuclear cells in such great numbers that it was impossible to interpret their presence except on the basis of their multiplying in the cytoplasm of these cells.

Recovery of B C G on artificial media met with only partial success. It was grown out on Herrold's egg medium in 2 cases from

fetuses inoculated with 3 mg. and from 2 fetuses injected with 1.5 mg. In twenty other attempts the bacilli were not recovered. The organisms did not seem to have changed in colony characteristics, morphology or staining reaction from this single passage. However, it did take a longer time for an abundant growth to appear, that is, 6 weeks as compared with 15 to 20 days for the original culture.

Fetus to fetus transfer of B C G was attempted on three occasions. Two of these are to be disregarded because the fetuses receiving the first subinoculation were aborted soon after injection. On the third attempt, however, three subinoculations were performed. The fetuses of 2 pregnant guinea pigs were inoculated with 0.1 mg. of bacteria. Three weeks later all the fetuses were removed under strictly aseptic conditions. The brain of one was ground up in a sterile mortar with 5 cc. of saline, lightly centrifuged and a smear made of the supernatant fluid. There were 10 acid-fast bacilli per oil immersion field. The following disposition of the material was made.

(1) 0.25 cc. was inoculated into the groin of each of 2 adult guinea pigs.

(2) Three tubes of Long's and one flask of Sauton's media were seeded.

(3) 0.1 cc. was inoculated into each of the fetuses of a pregnant guinea pig.

The adult guinea pigs killed and autopsied 6 months later showed no tuberculous changes although there had been a slight enlargement of the inguinal lymph nodes at first. No growth appeared on the media. The guinea pig whose fetuses had received the subinoculation was found 9 days later to be rupturing through the site of incision. An aseptic laparotomy was immediately performed and the fetuses removed. The brain of one was treated precisely as that of the initial fetus and the material used for the second serial transfer. However, no acid-fast bacteria were found in the supernatant fluid after centrifugation.

Adult guinea pigs inoculated with this material were negative for tuberculosis when autopsied 6 months after inoculation. No bacteria were recovered in culture. The fetuses were examined 3 weeks after inoculation but no evidences of tuberculosis were found. No tubercle bacilli could be demonstrated in histological sections of the fetuses from both the first and second transfers.

A third serial inoculation to adult animals, media and fetuses was made, again with negative results.

Summary: B C G was inoculated intracerebrally into fetal guinea pigs in doses ranging from 0.01 to 3 mg. The animals were examined for gross and microscopic pathology after removal by cesarean section or after birth. Most of the guinea pigs exhibited different stages of tuberculosis, depending on the dose and the time interval. A few of those receiving the smaller dosages manifested no evidence of tuberculosis. Many of the animals that were permitted to be born naturally died of generalized tuberculosis or showed extensive tuberculous changes when sacrificed.

Recovery of the organisms on artificial media was only partially accomplished and the few attempts at fetus-to-fetus transfer were unsuccessful.

TABLE I

Dissemination of Tubercle Bacilli in the Guinea Pig Fetus After Intracerebral Inoculation

Dosage	Fetuses inoculated with H37				Fetuses inoculated with B C G			
	Total	Number essentially normal	Number tuberculous		Total	Number essentially normal	Number tuberculous	
			Only at site of inoculation	In other organs also			Only at site of inoculation	In other organs also
mg. 0.01	13	0	0	13	5	4	1	0
0.1	12	0	0	12	13	1	7	5
1.0	7	0	0	7	14	1	2	11
1.4					4	0	0	4
1.5					3	0	0	3
2.0					10	0	0	10
3.0					7	0	0	7
Totals ...	32	0	0	32	56	6	10	40
Percentage showing dissemination of tubercle bacilli								
	100 per cent				71.4 per cent			

Discussion

Table I summarizes the incidence of dissemination from the site of inoculation of B C G, as compared with H_{37}. The latter bacillus was able to establish foci of infection in distant organs even with the smallest dosage. The B C G was able to spread too, but only when inoculated in the larger amounts, 0.01 mg. having hardly the ability to establish a local focus. However, too much weight is not to be placed on this fact since it was in the fetuses examined after the shorter periods that only local changes were found. Furthermore, it is the fetuses inoculated with the smaller dosages and allowed to go for the longer periods that fall into the last column of Table I. The gross and histological pathology produced by these agents in fetal guinea pigs was similar in all essential respects. In addition, the two types of bacilli in stained sections appeared morphologically identical.

Care must be exercised in judging the relative virulence of bacteria on the basis of histological evidence. The fact that B C G was found to spread from the site of inoculation is not sufficient to stamp it invasive. Even avirulent or dead bacteria inoculated by various routes may be scattered through the body by way of the blood stream. However, a certain degree of virulence and growth is implied when tubercle bacilli are associated with lesions showing various stages of development, are intracellular in large numbers, and assume a definite relation to one another extracellularly as well as intracellularly. Judged by these criteria, B C G seems to be definitely pathogenic for guinea pig fetuses. Furthermore, the similarity in the histological pictures produced by B C G and by H_{37} supports the same idea. Why B C G is relatively virulent for fetal guinea pigs and what the general significance of this fact is cannot be discussed here.

Our failure to pass B C G from fetus to fetus in the few attempts made appears to be inconsistent with the extensive pathological changes produced, yet it falls into line with work on adult animals reported by Feldman [3] and others.[10] A possible explanation lies in the factor of dosage since in the method employed there was no attempt to concentrate the inoculum.

The difficulty in recovering B C G on artificial media, also noted by Feldman,[11] perhaps may be explained by an insufficient

amount of inoculum. Control inoculations of the same media were made, using bacillary suspensions from cultures and containing the same number of organisms as in the fetal material subinoculated, that is, 10 acid-fast bacilli per oil immersion field. No growth took place, although tubes of the various media employed seeded directly with the dry organism showed abundant growth in 3 weeks.

It appears essential that one should be able to transmit B C G from fetus to fetus and to recover it on artificial media in order to establish its activity. More extensive experiments along this line are in progress.

Summary

Guinea pig fetuses were inoculated with graded doses of B C G and the effects compared with those obtained in a similar series inoculated with virulent tubercle bacilli (H37). Both types of tubercle bacilli were found to spread from the site of inoculation but not with equal rapidity; with the same dose, it took a longer time for B C G to cause as much pathology, in the animal as a whole, as H37. Pathologically the response of the fetus to both organisms was the same. Histological evidence has been brought forward to show that B C G is capable of multiplying in fetal tissues and of initiating a disease process different only in degree from that produced by H37. So far, attempts at fetus-to-fetus transfer of B C G have not been successful. Recovery of this bacillus on artificial media from inoculated fetuses was accomplished four times in twenty-four attempts.

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DESCRIPTION OF PLATE

PLATE 18

FIG. 1. Longitudinal section of brain of fetus 21 days after inoculation with 0.01 mg. of H37, Ziehl-Neelsen stain. Note tubercle occluding the foramen of Luschka and the resulting enlarged cerebral ventricle. Typical meningitis and inflammatory areas in walls of lateral ventricle. × 2.

FIG. 2. High power photomicrograph of meningitis in base of brain of Fig. 1, Ziehl-Neelsen stain. Typical granular tubercle bacilli. × 1500.

FIG. 3. Section of placenta from fetus 21 days after intracerebral inoculation with 0.1 mg. of H37, Ziehl-Neelsen stain. Early tubercle associated with older tubercle (not shown). Polymorphonuclear cells are present with the large mononuclear cells beginning to appear. × 1000.

FIG. 4. Section of brain of fetus 21 days after inoculation with 0.1 mg. of B C G, Ziehl-Neelsen stain. Large masses of acid-fast bacilli in the cytoplasm of large mononuclear reacting cells. × 1500.

FIG. 5. Section of brain from same fetus as in Fig. 4, showing acid-fast bacilli lying parallel extracellularly. × 1500.

FIG. 6. Section of brain of fetus 7 days after inoculation with 0.1 mg. of B C G, Ziehl-Neelsen stain. Typical large mononuclear cell lying free in lateral ventricle, the cytoplasm filled with acid-fast bacilli. × 1500.

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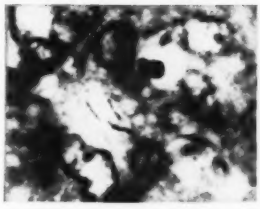

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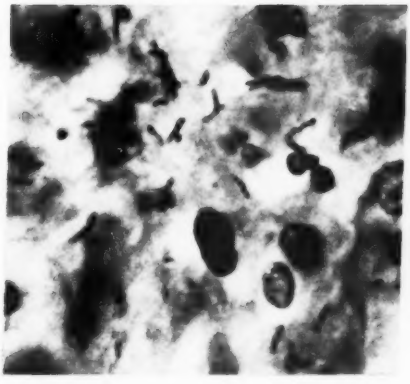

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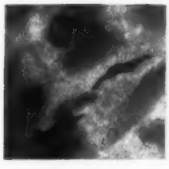

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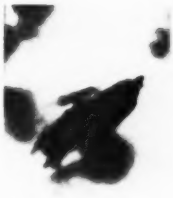

6

SUSCEPTIBILITY OF THE GUINEA PIG FETUS TO VACCINIA *

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INTRODUCTION

Vaccinia is one of a group of virus diseases that under natural conditions present lesions of similar appearance in a variety of animals. The experimental host-range of vaccinia itself is also wide, including man, monkeys, cattle, fowl and rodents. Animal susceptibility is not necessarily a property common to the members of an allied group, however, for the receptivity on the part of a single member may be of only a relatively mild degree. The response of the guinea pig to vaccinia is an example; while the rabbit is highly susceptible, the former is generally regarded as comparatively non-reactive.

The cultivation of the vaccine virus in tissue cultures has introduced a semi-artificial condition in which cells even of embryonal nature serve as a medium. The demonstration of this principle by Rivers [1] and his associates has been extended more recently by Goodpasture, Woodruff and Buddingh [2] to the successful cultivation of the virus in membranes of the chick embryo. They have found a greater susceptibility of the embryonic tissues than of the hatched chick to vaccinia.

Various agents capable of inducing disease have been reported as more invasive in avian embryonic tissues than in hatched or adult birds. As long ago as 1912, Murphy and Rous [3] reported that their chicken sarcoma grew more favorably in the embryo (chicken, pigeon, duck) than in the adult. The same has been found true by Rivers and Schwentker [4] in the case of a parrot disease virus. Mackenzie,[5] studying Rift Valley fever, Burnet,[6] working with canary pox, and Syverton, Cox and Olitsky [7] in equine encephalomyelitis experiments, likewise noted the growth of their respective viruses in chick embryo tissue cultures while young or adult fowl were not responsive.

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Woolpert,[8] in seeking to demonstrate any peculiar susceptibility of the fetus in a species whose adults are relatively resistant, found in preliminary experiments that the guinea pig fetus reacted to vaccinia virus. We have investigated the problem and this paper reports our studies on the susceptibility of this type of experimental animal to the virus. In short, it was found that lesions appeared, death sometimes ensued, and the virus increased in the fetal animal, while the same virus had little or no effect on the guinea pig after birth.

Experimental

Materials and Methods

The neurovaccine strain of Levaditi was used throughout these experiments. It was carried free of bacteria by testicular passage in rabbits, and its virulence, as measured by the ability to produce cutaneous lesions in the same animal, was constantly maintained. The operative technique was in general as described by Woolpert.[8] Most fetuses were inoculated intracerebrally, only a few intraplacentally.

About a third of the fetuses were injected with rabbit testicular material, the remainder receiving suspensions of guinea pig fetal tissues proved to contain virus. The potency of testicular and fetal virus was regularly determined by rabbit skin titration. The titer varied from 1:100 to 1:100,000, three-fourths the preparations having a titer of 1:10,000 and 1:100,000. The fetal virus usually measured 1:10,000 and the testicular material was somewhat more potent. The doses used were 0.1 cc. for each fetus inoculated, and when a preparation was injected in a number of fetuses it was commonly employed undiluted and diluted 1:10 and 1:100.

Fetuses in various stages of gestation were employed. The range was from 27 to 53 days of intrauterine life, the average was 39 and the median 37 days. The experimental period varied widely, being from 2 to 35 days; the majority of experiments were terminated between 5 and 7 days after inoculation. In addition to delivery of the fetuses by cesarean section, experiments were ended by such factors as threatened or actual abortion, birth, or fetal resorption. In a few instances the young that were born alive furnished other material

for examination. Excluding the fetuses that were not available for study because of early abortion, intrauterine maceration, resorption or calcification, and maternal destruction of aborted fetuses, data were obtainable on 115 fetuses inoculated with potent vaccine virus.

Controls on the virus employed in inoculation consisted of repeated demonstration of cutaneous vaccinial lesions in the rabbit and their prevention by mixture with antivaccinial rabbit serum. Furthermore, the results in the fetus were controlled by the recovery of the virus from fetal organs by rabbit skin inoculations and again its neutralization by specific immune serum. Occasionally a fetus of a litter was uninoculated and served as a control on the infectiousness of the material used in its litter mates. All operative procedures were carried out with strict aseptic technique and infrequent bacteriological examinations of the inoculum and the tissues of infected fetuses were negative.

Throughout the fetal experimentation the virus was repeatedly tested for its action on adult guinea pigs. The rabbit testicular virus was tested by the cutaneous and intracerebral routes, while the infected fetal tissues, controlled by rabbit skin reactions, were examined by the cutaneous, intracerebral, intravenous and intratesticular routes. The only observable lesion elicited in the guinea pig was a mild hyperemia with papule formation at the site of intracutaneous inoculation. This appeared only occasionally and with the highest concentration of inoculum. In no instance was a febrile reaction obtained. These results indicate the relative non-reactivity of the adult guinea pig to the test virus employed in the fetal experimentation and furnish a basis for comparison with the results to be described.

Results

The action of the virus on the fetus could best be judged by the gross and microscopic pathology. Death as a criterion of virus effect was not dependable, since fetal mortality may result from natural causes and we are not aware of its incidence under ordinary conditions. In our experimental series dealing with 80 mothers there were 10 fetuses that were found in various stages of resorption, dehydration and calcification. This is not a complete picture, however, as no doubt many killed by virus action were aborted and lost.

Gross Pathology

The lesions seen in the infected fetuses appeared in the skin and various viscera. Because of the small size of the organs, the presence of lesions was judged mainly by the external changes in each part. For the same reason, pathology in the intestines was not definable. Changes were recorded in various parts of 88 of 115 fetuses inoculated with virus proved potent by rabbit skin test and observed under conditions suitable for detection of lesions. The 27 fetuses not showing any gross pathology included those injected with high dilutions of virus material. Of the 88 animals, lesions were seen in the skin in 33, on the surface of the brain in 11, in the lungs in 54, in the liver in 25, in the kidneys in 55 and in the spleen in 9 instances. The placenta and heart showed gross changes in each of 2 fetuses. It is clear that the distribution of lesions was irregular and that they occurred most commonly in the lungs and kidneys.

The lesions in most parts were circumscribed and usually with a regular margin. They varied in size from being barely discernible to the naked eye to 2.5 mm. in diameter. The largest number of lesions were flat, although a few were elevated in the form of vesicles and some were depressed or pitted. The kidneys of 3 fetuses were marked by small ulcerating foci. Most of the lesions were gray or gray-white and with a sharp outline; these occurred in all the tissues but were most conspicuous in the skin. Minute hemorrhagic areas appeared on the lung surfaces in 11 fetuses, and occasionally irregular areas of hemorrhage 2 mm. in diameter surrounded by diffusely pale grayish tissue were found involving the kidney. Rarely, gray lesions elsewhere were tinged with red or surrounded by narrow zones of hemorrhage. In general, no differences in the gross changes in individual sites were attributable to the source of virus, although lesions of the brain were more common in the fetuses inoculated with rabbit material, and splenic alterations were seen only in the animals that received fetus virus. Representative lesions are pictured in Figures 1 to 7.

Microscopic Pathology

The microscopic features characterizing the acute effect of vaccine virus on the fetal tissues are necrosis, edema and hemorrhage. There is a noteworthy lack of cellular infiltration, although in older lesions

a mesenchymal reaction is apparent. The detailed study of the histopathology will be made the subject of another communication.

Vaccinia in the Fetus

Thirty-three of the inoculated fetuses were examined in detail for the effect of vaccinia. Suspensions of their tissues were reinoculated into rabbit skin for the identification and titration of virus and to determine its distribution in the fetus. Twenty-six had been injected with suspensions of infected fetal organs (15 with renal, 7 with brain, 2 with lung and 2 with placental suspensions) and 7 received rabbit testicular virus. The results cannot be correlated with the type of material injected and the data will be considered together. Further, the titer of the virus in the organs examined was not referable to the strength of the original inoculum. The period varied widely, as previously indicated; the tissues of 22 of these fetuses were examined from 4 to 7 days after inoculation. The duration of the experiment under these conditions did not seem to have a direct bearing on the demonstration of virus and its distribution and titer.

Vaccine virus was recovered from fetuses by rabbit inoculation and the rabbits showing positive skin reactions to this virus were proved immune to subsequent infections of virus of rabbit origin. Virus was demonstrated in all but 3 of the 33 fetuses, and in 2 of these, lesions in the lungs were apparent. Virus was recovered from 18 of 24 brains, 20 of 31 lungs, 13 of 26 livers, 6 of 17 spleens, 23 of 26 kidneys, 2 of 3 skin lesions, and 11 of 17 placental tissues tested. The blood of 10 fetuses was examined and only 2 yielded virus and they in low dilution. In all, suspensions of 146 organs (excluding blood) were tested quantitatively in tenfold dilutions. Ninety-four gave positive results in the following titers: 13 to a titer of 1:1; 10 to 1:10; 36 to 1:100; 15 to 1:1000; 18 to 1:10,000; and 2 to 1:100,000. Not only did the kidney furnish the highest incidence of virus recovery, but it also more often gave the highest titer among the organs, usually being 1:10,000. It was used most frequently as a source of passage virus.

When the incidence of virus recovery was compared with the presence of lesions in various parts, it was observed that of the 146 tissues examined for virus, lesions and virus both were found in 57 tissues, neither was in 37, the virus and no lesions were in 39, and

lesions but no virus were demonstrable in 13. In this series of 33 animals, gross changes were thus noted in 70 tissues. In 3 fetuses no pathology was seen, but in 2 of them the virus was isolated from some part. The virus was found to persist to the limit of the experimental periods and was recovered as late as 32 days after fetal inoculation. Likewise, lesions were observed after the maximum interval of experimentation.

Fetal Source of Virus

We have already indicated that the virus of fetal origin behaved in fetuses as that of rabbit passage in all the respects noted in the experiments. That the virus increased in the fetus was demonstrated by the effective transfer of virus through series of fetuses. In one group the fetuses of 9 mothers were serially inoculated, beginning with 1: 100,000 titer of testicular neurovaccine and ending with 1: 10,000 titer of virus in the fetal kidney, with lesions present. Besides establishing the multiplication of vaccinia virus with the development of lesions in the fetus, it was found that the highest concentration and the most frequent effect of the virus was in the kidneys. With all this, however, there seemed to be no selective adaptation of the virus to the fetus, since the virus was constantly transmissible to the rabbit with typical manifestations, its characteristics in the guinea pig fetus did not change during the year of experimentation and it was not modified in its relative non-infectivity for the adult guinea pig.

Vaccinia in the Newborn

All gradations of effects were noted among the 35 animals that were delivered at term after inoculation *in utero*. Six died soon after birth and some displayed lesions with virus demonstrated in low titer in rabbit skin. Nine others lived from a few days to 3 months; of these, 2 had vaccinial lesions in the lung and kidney, 3 showed softening of the cord and brain after symptoms of paralysis and weakness, and the others died showing pathology not referable to experimentation. The remaining 20 animals survived. Although the skin in all animals born was examined thoroughly, often from the undersurface as well, no lesions were seen in the group that was born alive.

The susceptibility of the newborn was tested, with the same non-reactivity resulting as was obtained in the inoculation of adult guinea pigs. Eleven animals from 4 to 10 days old were injected intracerebrally with potent virus and none showed fever or symptoms.

Discussion

Much experimental work has been done on the parasitization of mammalian hosts with viruses. The range of animals in which disease is induced has been greatly extended by the introduction of special techniques, as well as by the use of animals not naturally exposed to specific infection. Little has been done, however, on the susceptibility of the mammalian fetus. The whole question of fetal reactivity has been only slightly explored, due perhaps to hesitancy in operative procedures. Although there are definite restrictions on account of the essential circumstances, the field is worth exploring on the bases of parasitism and fetal physiology.

We are here reporting the successful propagation of the virus of vaccinia in the guinea pig fetus, with the development of typical lesions. This prenatal susceptibility, as compared with the relative resistance of the adult, is consistent with the work already cited of the successful growth of viruses in cultures of embryonic tissues. Such cultures apparently furnish a circumstance sufficiently favorable for intracellular growth. That this is possible in tissue cultures of the natural host is logical, but evidently some other factors enter that allow virus growth in the fetus whose adult is relatively insusceptible. This may be due to the fact that the fetus furnishes a different type of tissue marked by the two properties of comparative immaturity and rapidity of cellular growth. The principle of virus increase in rapidly growing cells may be thought of as the reverse of the property of viruses to stimulate host cell growth, as pointed out by Rivers [9] in connection with infectious myxomatosis of rabbits, and as observed to be an essential result of viruses inducing tumor growths.

The microscopic examination of affected tissues, so far as it has been carried, does not disclose a marked cellular reaction. It might be supposed that fetal susceptibility to vaccinia depends on this relative lack of cellular response. That the fetus reacts to some infectious agents with the proliferation and infiltration of inflamma-

tory cells is illustrated by the descriptions of the response of the fetus to the tubercle bacillus [10] and to the submaxillary gland virus.[11] It thus appears that the infectivity of the fetus does not depend on the absence of demonstrable cellular defense. Susceptibility is based on some other type of cellular reactivity, as in postnatal animals, and the relative susceptibility in this case is apparently referable to the properties of fetal cells already mentioned. On the other hand, the lack of universal susceptibility, as pointed out by Woolpert,[8] furnishes a situation equally inexplicable.

Most fetuses were inoculated intracerebrally. The virus content of brain tissue, however, was found to be low at the termination of the experimental periods, much lower in fact than in distant organs. The kidneys, and to a less degree the lungs, were sites of predominant virus localization. The predilection of the skin for pock development is significant in view of the dermal nature of the virus. Judging by the wide distribution of lesions and by the high virus titer of tissues distant from the site of injection, it appears that the fetal guinea pig is parasitized by vaccinia virus in a general way, much like generalized vaccinia in the rabbit and in the chick embryo in the shell, as described by Goodpasture and Buddingh.[12] The virus content of tissues was not dependent, however, on its supply of infected blood, since the blood contained demonstrable virus only twice in ten specimens and then only in low dilutions.

Necrosis was the chief pathological finding. Areas involved were mainly circumscribed with, in the skin, the development of a definite pock. Superficial lesions in the viscera were likewise sharply outlined, except in the kidney where blanched areas indicated a more diffuse process. Hemorrhage occurred frequently in the center of necrotic lesions and sometimes constituted the main gross alteration in the lungs. The lesion in the fetus reached a peak macroscopically in 3 to 5 days and did not seem to progress appreciably during the remainder of the experimental period. A distinct disadvantage in this work was the inability to observe the development of lesions.

The use of vaccine virus propagated in tissue culture and in the chick embryo in the shell has been proposed for human vaccination purposes. Virus of fetal guinea pig origin might be useful to the same end because of its bacterial sterility and high potency, but much must yet be done to standardize and regulate its production.

It should be pointed out that we were unable, by repeated fetal passage, to modify the relation of the virus toward the postnatal guinea pig, on the one hand, and toward the rabbit, on the other. After as many as nine transfers from fetus to fetus, the virus still was ineffective when injected into young or adult guinea pigs by any route and yet retained its ability to induce typical lesions in the rabbit by skin or testicular inoculations. This points to the stability of the virus under these conditions, a property that has been commented on by Goodpasture and Buddingh.[12]

Summary and Conclusions

The vaccine virus was successfully propagated in the fetal guinea pig. The effect of the virus was to induce typical lesions in various organs and tissues. The same virus strain caused only the slightest reaction in the postnatal animal. These results indicate a markedly greater susceptibility of fetal tissues for an infectious agent. The cultivation by other workers of certain viruses in embryonic tissues, when adults of the same species were not reactive, points to the same phenomenon. The mechanism of this comparative effect is not yet clear, although general immaturity and rapidity of cell growth in the fetus are suggested as possible factors.

Lesions were found irregularly distributed in the principal organs, including the skin, and were most common in the lungs and kidneys. Of all the fetal tissues the kidney yielded virus most constantly and in the highest titer, usually measuring 1: 10,000.

The vaccine virus was passed through a series of fetuses and was not appreciably modified in its cutaneous activity in the rabbit or in its failure to induce effects in the postnatal guinea pig.

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DESCRIPTION OF PLATE

PLATE 19

FIG. 1. Fetus delivered dead by cesarean section 6 days after intracerebral inoculation with 0.1 cc. of 1:10 dilution of tissue culture vaccine virus. Multiple, circumscribed cutaneous lesions, some pitted. Patches of adherent fetal membrane around left eye and over scalp. × 1.

FIG. 2. Fetus delivered alive by cesarean section 10 days after intracerebral inoculation with 0.1 cc. of 1:10 dilution of rabbit testicular vaccine (titer 1:1000). Round gray lesions on eyelid, back, side, heel (not in focus), placenta and in adhesion of fetal membranes. Fetus still enclosed in amniotic sac. × 1.

FIG. 3. Lung of fetus delivered alive by cesarean section 7 days after intracerebral inoculation with 0.1 cc. of 1:100 dilution of rabbit testicular vaccine (titer 1:10,000). Diffuse gray areas with hemorrhagic centers on diaphragmatic surface. × 2.

FIG. 4. Lung of fetus of Fig. 2, showing two gray lesions on costal surface. × 1.5.

FIG. 5. Skin lesion of fetus of Fig. 2, in patch of white hair. × 1.5.

FIG. 6. Diffuse necrotic areas in upper pole of left kidney and in both poles of right kidney of fetus of Fig. 2, with hemorrhagic centers in lesions of both upper poles. (White areas are recognized as bits of fetal fat.) × 1.5.

FIG. 7. Two lobes of liver (others removed) of fetus of Fig. 2, showing ten minute gray lesions on surface. × 1.5.

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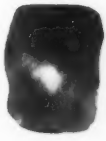

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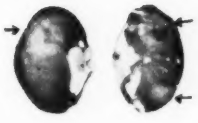

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Stritar and Hudson Susceptibility of Guinea Pig Fetus to Vaccinia

SUSCEPTIBILITY OF THE GUINEA PIG FETUS TO THE SUBMAXILLARY GLAND VIRUS OF GUINEA PIGS*

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INTRODUCTION

Experimental work with the submaxillary gland virus, in the absence of other susceptible animal species, is limited to the natural host. Since proportions varying from 30 to 84 per cent of stock animals have been found infected, and therefore immune, it has been necessary to study the virus in young animals 2 to 3 weeks old. Guinea pigs of this age are seldom spontaneously infected, but in our study of brain to brain transmission of the virus [1] we found considerable variation in susceptibility, even among these young animals. We have since determined that newborn guinea pigs 1 or 2 days old respond more uniformly to intracerebral injections of the virus. The development of the technique of fetal inoculation in this laboratory by Woolpert [2] placed at our disposal a new method for studying the virus, which practically excludes the possibility of interference from spontaneous infections. The present paper deals with the submaxillary gland virus infection in the fetus and the factors and influences associated with it.

EXPERIMENTAL

Methods and Materials

The virus used for fetal inoculations was obtained from the salivary glands of guinea pigs supplied by local dealers or of stock animals artificially infected by ourselves. Virus emulsions for fetal injections were prepared in the following manner: the submaxillary glands were aseptically removed from 3 or more adult guinea pigs and ground in a mortar with sterile sand. The pulp was taken up in saline solution to the amount of 1 cc. for each gland. Gross particles were allowed to settle out or were thrown down by light

* Received for publication September 30, 1935.

centrifugation. The supernatant fluid was considered a stock emulsion for purposes of making dilutions.

The fetuses of pregnant guinea pigs in the 4th or 5th weeks of gestation were most suitable for inoculation. Injections were made intracerebrally or intraplacentally in doses of 0.1 cc.

Results and Pathology

The present paper is based upon a study of the tissues of 60 fetuses of 20 mothers. Experiments were terminated at intervals varying from 3 to 21 days after inoculation. When a litter was composed of 3 or more fetuses, 1 of them was usually left uninoculated as a control. In addition to the various fetal tissues, the submaxillary glands, kidneys, spleen and sometimes the lungs of the mother were studied histologically.

In fetuses that were delivered by cesarean section before they succumbed to the infection, the gross changes generally consisted of petechial hemorrhages in the subcutaneous tissues and viscera, discrete necrotic foci in the liver, and enlargement and infarction of the placenta. Death of the fetus *in utero* was followed within 24 hours by generalized edema and subsequent dehydration. The latter process ultimately reduced the fetus to a blackened, wax-like mass. The placenta in such cases was usually diffusely necrotic, but on two occasions we found it to persist in a fairly healthy condition when the fetus was partially resorbed.

In general, it may be said that the course of the infection in the fetus is governed by two reciprocal factors: the interval elapsing after inoculation and the potency of the inoculum. The influence of the latter factor on the gross pathology in the fetus may be illustrated by the following results of a titration of virus in a gland emulsion. Dilutions of 1: 10, 1: 100 and 1: 1000 were prepared in the manner described above and injected cerebrally into the 3 fetuses of 1 guinea pig. On the 8th day after inoculation the mother was anesthetized and the inoculated animals delivered by cesarean section. The fetus receiving the 1: 10 dilution weighed 6.5 gm. and was shrunken, black, and of a wax-like consistence. It had been dead approximately 5 days and its placenta was pale and necrotic throughout. The fetus injected with the 1: 100 dilution weighed 12 gm. and was pale, soft and somewhat dehydrated. The placenta contained

both bland and hemorrhagic infarcts, but about half of the tissue was normal in color and texture. This animal had been dead approximately 3 days. The third fetus which had received the 1:1000 dilution weighed 16 gm. and was swollen and gelatinous in consistence and the tissues were blood-tinged. Numerous petechial hemorrhages were present in the subcutaneous tissues and viscera. The placenta was somewhat enlarged but otherwise normal in appearance. This fetus had probably died less than 24 hours before delivery.

It will be noted that the degree of change in each fetus was proportional to the dilution of the inoculum it received and to the length of time it survived after inoculation. In our experience this proportionality was generally very regular when potent virus emulsions were titrated. When weak suspensions were tested the gross changes were much less obvious, but histological examination of the fetal tissues revealed a series of changes that enabled one to reconstruct the course of events leading to the death of the fetus.

So far as we are aware, no other known disease of the guinea pig is accompanied by the presence of intranuclear and cytoplasmic inclusions which might in any way be confused with those of the submaxillary gland virus. Negri, Guarnieri and Bollinger bodies have long been considered important differential and diagnostic aids. In the same way, we have adopted the presence of characteristic intranuclear inclusions in the mononuclear cells as a histological criterion of infection in the fetus. These distinctive cells are associated with all of the lesions in the fetus and are therefore invaluable in studying the course of the infection and assessing the significance of tissue alterations.

Intracerebral introduction of the virus in the fetus was regularly followed by a mononuclear meningitis (Fig. 1), and in severe infections by hemorrhage and liquefaction of the brain. Although the meningitic reaction is most intense about the larger vessels, particularly those of the longitudinal fissure and the ventral surface of the brain, most of the inflammatory cells appear to be of local mesenchymal origin rather than an infiltration of blood-borne mononuclears. Large eosinophilic inclusions are in the nuclei of many of the reacting cells. Unlike the infection in the newborn guinea pig in which the lesions are primarily confined to the meninges, extensive changes are usually found in all the fetal viscera and placenta. In

the liver two kinds of lesions are found: necrotic foci in the parenchyma and perivascular infiltrations and proliferation in the portal triads (Fig. 3). The necrotic foci vary considerably in size and number and do not occur in the absence of the periportal lesions. The intensity of the latter change appears to vary with the extent of the infection in the meninges and placenta. The infiltrating cells consist of myelocytes and non-granular leukocytes. Inclusions are not found in the former cell type, but the mononuclears do become infected and when present in sufficient numbers form a necrotic focus which involves the vascular endothelium.

Following cerebral introduction of the virus, its localization in the placenta is accompanied by proliferation of the local mesenchyme, and in severe infections by thromboses of both the maternal and fetal blood channels. The greatest number of inclusion-containing cells is usually seen in the placental labyrinth (Fig. 2). Perivascular reactions sometimes progress to the point of obliterating the smaller fetal vessels. Where such affected vessels are embedded in the chorionic syncytium and close to the free border bathed by maternal blood, leukocytes from the maternal blood sinus penetrate the epithelium and invade the infected fetal mesenchyme. In some instances a break in the placental barrier was accompanied by thrombosis of the maternal sinus. The size, number and location of the infarctions caused in this manner, and the extent of the vascular disorganization and damage in the liver, seem to determine the survival of the fetus. The remarkable resistance of the chorionic epithelium to infection and injury when surrounded by masses of infected fetal mesenchyme is a striking feature of the placental pathology.

When virus is injected into the placenta instead of intracerebrally, changes occur in both the liver and the placenta similar to those described above. The injected placenta may be double the weight of that of an uninoculated litter mate. This increase in weight is largely due to the infection and proliferation of the placental mesenchyme. It is of interest that "the dome of the central excavation," which is of mesodermal origin, has never been found infected.

In addition to the lesions in the meninges, liver and placenta, small foci usually associated with recent capillary hemorrhage were found in the brain, lungs, spleen, thymus and in the subcutaneous tissues. A number of infected macrophages were once found in a slight reaction in the subcutis overlying an erupting tooth. Inclusion bodies

were always confined to the mesenchymal cells or to tissues derived from the mesoderm; in no instance were they found in epithelial or nerve cells.

Immunity

It might be supposed that the presence of spontaneous infection in the salivary glands of the mother and her consequent immunity would alter the susceptibility of the fetus or the course of the infection in it. In spite of the fact that the placenta of the guinea pig is of the hemo-chorial type (Grosser's classification) and thus affords optimum conditions for the passive transfer of humoral antibodies, we have found no evidence that the immune state of the mother affected the susceptibility of the fetus or the course of its infection. The glands of 12 mothers whose fetuses were artificially infected were examined histologically and 10 of them contained evidence of long-standing infection. It is of interest in this connection to compare the susceptibility of the prenatal with that of the postnatal animal. Young guinea pigs varying in age from 12 hours to 2 weeks succumbed to intracerebral injections regardless of whether their mothers were spontaneously infected, or whether the young were allowed to suckle immune mothers. It appears from these observations that passive transfer of antibodies, either prenatally, or postnatally with the milk, does not occur in appreciable amounts. These findings are in accord with those of Kuttner [3] who was unable to satisfy herself as to the presence of neutralizing antibodies for this virus in the serum of immune adults.

Further evidence of the inferior or transient nature of the immunity associated with submaxillary gland virus infections was furnished by another type of observation. In this instance the fetuses were inoculated intracerebrally. The infection metastasized to the placenta where maternal leukocytes infiltrated and broke down the placental barrier. Infected fetal macrophages were seen in the maternal blood sinuses. The renal epithelium of the mother was extensively and recently infected, as indicated by the presence of inclusions in the epithelial cells and the absence of cellular infiltrations about the affected tubules. Examination of the mother's salivary glands revealed a single focus of infection which was heavily infiltrated with lymphocytes and in which the inclusions stained very poorly. Cells with inclusions in them have been observed in the lungs of other

mothers when there have been breaks in the chorionic epithelium and inclusion-laden cells in the maternal blood sinuses. In these mothers, however, there has been no evidence of superinfection if their salivary glands contained recent or active lesions.

Discussion

It is necessary to recognize two important factors in comparing the susceptibility of the fetus with that of the newborn and young guinea pig to the salivary gland virus. The first of these is the ability of the fetus to survive when the brain is partially or totally destroyed. As long as its placenta and cardiovascular system are relatively intact, it can withstand almost unlimited insult; this is not true of the postnatal animal. Thus the susceptibility of the two animals cannot justly be based solely on the period of survival after inoculation.

The second important factor is the greater functional development of the defensive mechanism, as regards the submaxillary gland virus, in the young guinea pig as compared with that of the half-term fetus. Actual details of the development and operation of this mechanism are lacking, but it appears that humoral antibodies are not involved to any appreciable degree. However, the marked cellular response to intracerebral inoculation in the young guinea pig is in sharp contrast to that in the fetus, and may be significant. In the former animal intracerebral inoculation of virus is followed by a considerable exudate of lymphocytes, as well as by infection of the local mesenchymal elements. The lymphocytes do not contain inclusions and inclusion-laden mononuclears are never seen in the blood vessels, although the salivary glands may become infected when the incubation period lengthens to 15 or 18 days — an indication that virus does escape from the central nervous system. In the fetus, on the other hand, there is almost no cellular exudate in the meninges even after cerebral inoculation, and infected mononuclear cells may be seen in the blood vessels and tissues in numerous parts of the body.

The difference in the extent of the infection in the prenatal and postnatal animal does not seem to be adequately accounted for by a difference in tissue susceptibility, for it has been shown by Cole and Kuttner [4] that when virus is injected into such sites as the lung, the testes or the tongue of young guinea pigs, inclusions are formed in

the local mesenchymal cells. It is problematical whether the presence or absence of a meningeal exudate is the important limiting factor or whether the difference in tissue susceptibility is one of degree rather than of category. However, it may be said that as a test animal for the presence of the virus the fetus is superior to the postnatal animal. Amounts of virus that cause not so much as a rise in temperature in young guinea pigs will, in the fetus, proliferate and ultimately bring about its death. In addition, when large amounts of virus are required, placental inoculations produce a far richer supply in fetal tissues than can be obtained from postnatal guinea pigs, either spontaneously or artificially infected.

Summary and Conclusions

Infection of the guinea pig fetus with submaxillary gland virus is described.

Typical inclusion bodies were found and were taken as an indication of the presence of the virus. On this basis the virus was apparently generalized throughout the fetus. Lesions appeared in most of the organs, especially the brain, liver and placenta. When death occurred, it seemed to be due to interference with the circulation by lesions in the placenta and liver.

It was determined that the fetus is better suited in many respects for experimental work with the submaxillary gland virus than the newborn or young guinea pig. Interference from spontaneous infections is avoided, smaller amounts of virus can be detected and the yield of virus is greater.

The susceptibility of the fetus is not altered by the immune state of the mother.

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DESCRIPTION OF PLATE

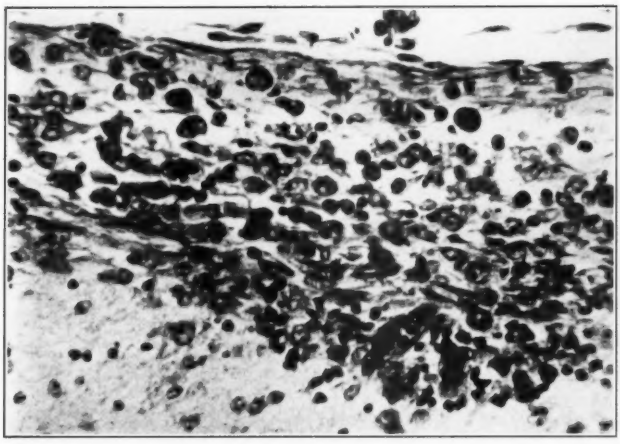
PLATE 20

Photomicrographs of paraffin sections stained with eosin-azure. Wratten-Wainright green filter "B" employed.

FIG. 1. Meningeal reaction following intracerebral inoculation of submaxillary gland virus in a fetus 5 weeks old. Slight involvement of the superficial cortex and extravasation of erythrocytes are shown. × 250.

FIG. 2. Placental infection following intracerebral inoculation of virus. Several inclusion-laden mesenchymal cells are shown in the fetal labyrinth. × 1000.

FIG. 3. Perivascular reaction in portal area of fetal liver following intracerebral injection of virus. × 250.



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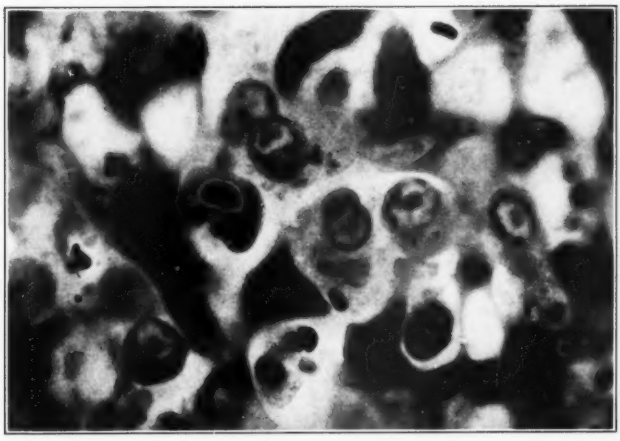

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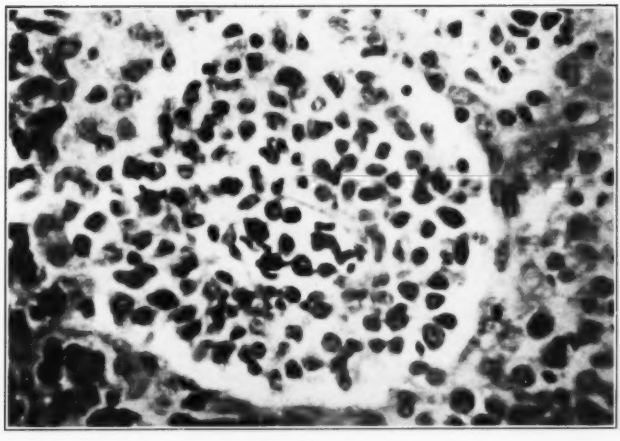

3

PERICARDIAL LESIONS IN RHEUMATIC FEVER *

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The tendency to affect serous membranes is one of the most characteristic features of rheumatic infection. Because of the striking appearance of pericardial inflammation in rheumatic fever its presence was frequently noted by early writers, who stressed its significance as evidence of cardiac disease. In 1761 Pulteney [1] reported a case of adherent pericardium in a young man who had suffered from fever and arthritis 2 years earlier and who died with signs of heart failure. Baillie [2] (1793) first described the acute form of pericarditis, with exudation of "coagulable lymph" from blood vessels in the pericardium and fluid in the pericardial cavity. He also recognized the thickening, congestion and adhesive tendency of the membrane, the conversion of the pericardium into cartilage, and the fact that pericarditis could be survived. A completely adherent pericardium in a 14 year old girl who died of acute rheumatic fever was reported by Wagstaffe [3] in 1803. Similar cases were reported by Crowfoot [4] and by Wells.[5] The former described 1 case with ossification of the adherent pericardium. The data on the case, however, are insufficient to establish a rheumatic etiology of the lesion.

By the beginning of the nineteenth century textbook writers were describing gross pericardial lesions as lucidly and accurately as today. The reticulated, curdled appearance of fibrinous pericarditis was likened by Corvisart [6] to the internal surface of the second stomach of a calf, and by Laennec [7] to "bread and butter," an expression retained by Hope [8] and all textbook writers after him. Laennec outlined the sequence of events in pericarditis as beginning with redness of the pericardium in isolated plaques, continuing with an albuminous exudate covering the entire surface, and concluding with healing and the resulting pericardial adhesions. He either did not recognize or did not stress the etiological significance of acute rheumatic fever.

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Although the etiological relation between pericarditis and rheumatic fever had already been noted in isolated cases, credit is generally given to Bouillaud (1840) [9] for conclusively demonstrating clinically and pathologically, in a large series of cases, the constant association of pericarditis, endocarditis and rheumatic fever. Yet in 1808 Dundas,[10] in 9 cases, had recognized pericarditis as occurring at the beginning or termination of one or more attacks of rheumatic fever. Of the 6 cases in which there was a postmortem examination, 1 had a pericardial effusion and the others an adherent pericardium. Likewise, Andral [11] in 1826 fully recognized the relation of 4 cases of pericarditis to acute rheumatic fever. Hope [8] in 1832 pointed out the importance of the latter as a cause of both acute and chronic pericarditis. He described the characteristic shaggy, flocculent, rough-patterned gross appearance of pericarditis and analyzed the three essential features of the acute disease as unusual redness of the membrane, "coagulable lymph" adhering to the surface and fluid effused into the cavity. He realized that the frequently resulting adherent pericardium, although a healing process, produced organic disease which might be fatal.

Very little has since been added to these gross descriptions of rheumatic pericarditis. The earliest studies of the microscopic appearance of pericardial lesions were made in 1890 by Krehl,[12] who in 8 cases of pericarditis associated with valvular heart disease described round cell infiltrations, especially about the smaller blood vessels, and intimal sclerotic changes in the larger blood vessels. But since some of these cases were undoubtedly not of rheumatic etiology and most of them patients in the fifth decade of life or beyond, the significance of these findings is somewhat lessened. More detailed and accurate microscopic descriptions followed the recognition of the specific Aschoff nodules as a rigid pathological criterion of rheumatic heart disease. Coombs [13] was the first to note them in the pericardium in 4 cases of acute rheumatic fever. They occurred only in the visceral pericardium, singly or in groups, and, perhaps because of freer surroundings, more loosely arranged than the Aschoff bodies in other cardiac tissues. Wätjen [14] described a case with Aschoff bodies in the walls of the small branches of the coronary artery. Mönckeberg [15] reported 2 cases of rheumatic pericarditis with Aschoff bodies in the pericardium, in 1 of which they were macroscopically visible as grayish, tubercle-like nodules over the right

ventricle and auricle. Sacks [16] also observed that they were often quite large and were found most frequently during the period of organization of the pericarditis.

More recent descriptions have included the microscopic appearance of the acute exudative phenomena. Coombs [13] noted endothelial proliferation, degeneration and desquamation. The raw surface was covered with fibrin. New capillaries and cells in the visceral pericardium, uniting with similar structures in the parietal layer, formed adhesions. Below the endothelium there was a zone of leukocytosis, chiefly mononuclear, and a layer of new connective tissue and capillaries. In a similar description by Bronson, Carr and Perkins,[17] grossly there was fusion of the thickened parietal and visceral layers, readily distinguishable and bound together by a third central layer of edematous connective tissue. Microscopically the outer parietal layer was composed of dense hyaline connective tissue covered superficially by a looser vascular fatty tissue, the inner or visceral layer of loose areolar tissue with many strands of compact hyaline connective tissue injected with small blood vessels, and the third central layer of edematous granulation tissue with occasional irregular masses of fibrin. Throughout, but particularly in the central and visceral layers, there was a marked infiltration of leukocytes, with a predominance of lymphocytic and plasma cells. In the central layers there were numerous polymorphonuclear leukocytes and endothelial cells, some of which were multinucleated.

Swift [18] emphasized the similarity in reaction of the pericardium and of other tissues in rheumatic disease. He stressed the difference between the non-specific extensive serofibrinous reaction, which was not significantly different from the response of other lining membranes to extensive injury, and the more localized granulomatous rheumatic lesions that represented a specific response to a rheumatic infection.

Sacks [16] considered fibrinous pericarditis one of the most distinctive lesions of rheumatic fever. He described the process as beginning with fibrin deposition followed by serous effusion and the exudation of leukocytes and erythrocytes. There was also desquamation of serosal cells. These, however, persisted below the fibrin for some time. The subpericardial tissues were edematous, containing numerous capillaries and fibroblasts and there was a diffuse cellular reaction with concentration around the small vessels. The blood

vessels showed endothelial swelling and proliferation, sometimes with thrombus formation. In a careful study of 250 serial sections from the heart of a case of acute rheumatic fever Darré and Albot [19] distinguished the fibrinous exudate on the surface, the reaction of the pericardial serosa proper and that of the subepicardial fatty tissue. Recently, Klinge [20] emphasized the similarity of rheumatic pericarditis to the rheumatic reaction of other cardiac structures and pointed out that the first stage was not the deposition of fibrin on the surface, but the same type of "fibrinoid" swelling of the ground substance of connective tissue as occurs elsewhere in the heart.

Peculiar polypoid and cyst-like formations were found by Lauche[21] in a case where they were probably due to desquamated epithelium persisting in the pericardial sac, fused at their tips and enclosing hemorrhagic exudate, and by Bohrod [22] in a case where, though more solid, they probably represented similar cysts whose original fluid content had become organized.

As the above review indicates, previous descriptions of the microscopic features and sequence of events have been presented in isolated cases, or as incidental findings in more detailed studies on other cardiac structures. We have, therefore, undertaken a detailed and systematic investigation of the pericardial lesions in a large series of cases of rheumatic heart disease which came to postmortem examination.

Material and Methods

There were 87 cases ranging from 17 months to 67 years of age. Of these, 68 presented myocardial Aschoff bodies and other evidences of active rheumatic infection (Rothschild, Kugel and Gross [23]). The remaining 19 presented the characteristic valvular deformities and old auricular lesions of inactive rheumatic fever. No Aschoff bodies were present in these inactive rheumatic cases. The first mentioned 68 cases were segregated into four groups according to the classification outlined by Gross and Ehrlich: [24]

Group I. Active cases in which the first attack was fatal (10 cases).

Group II. Active cases in which there was one attack prior to the final fatal recurrence (18 cases).

Group III. Active cases with repeated attacks and death during an acute recurrence (12 cases).

GROUP IV. Active cases in which death occurred with signs of congestive heart failure but without clinical evidence of an acute rheumatic recurrence (28 cases).

The remaining 19 cases of clinically and pathologically inactive rheumatic fever will be referred to as Group V. The purpose of this classification is to correlate the pathological findings with the clinical course of the disease.

The normal histology of the pericardium was studied in 35 additional cases evenly distributed between the ages of 3 months and 74 years, without clinical or pathological evidence of cardiac disease. Standard sections of both normal and abnormal pericardiums were made according to the technique described by Gross, Antopol and Sacks.[25] The detailed methods of fixation and the stains employed have been described elsewhere.[26] After fixation and cutting, the parietal layer of pericardium is not visible on microscopic section except where it has become adherent to the visceral layer because of inflammation. Our microscopic descriptions of both the normal and abnormal pericardium, therefore, will generally refer to its visceral layer or epicardium.

GROSS PERICARDIAL LESIONS

While the gross changes in rheumatic pericarditis have been adequately described before, a few comments as to the incidence, nature and distribution in the larger series of cases which we have studied appear pertinent, particularly with reference to the above clinical classification.

In general, there was a much higher incidence of gross pericarditis in the cases where death appeared clinically to have taken place in an attack of acute rheumatic fever, *i.e.* in the first three groups. Of the 10 cases in Group I in which death presumably occurred in the first attack, 6 had definite gross pericarditis consisting of a fibrinous exudate over the entire pericardium with thickening, glazing and adhesions. In 3 of these there was also a variable amount of straw-colored turbid fluid containing many shreds of fibrin. In 1 of these the exudate was definitely fibrinopurulent. A 7th case showed a localized patch of fibrous thickening over the right auricle.* The remaining 3 appeared to have a normal pericardium.

* The so-called "milk patches" were disregarded because of their uncertain and variable etiology.

In Group II, where one attack preceded the final one, there was an even higher incidence of gross pericarditis. Fourteen of the 18 cases (78 per cent) showed a universal pericarditis, consisting generally of the thick, shaggy, bread-and-butter type of lesion with numerous fibrous adhesions. Seven of the 14 had a completely adherent pericardium with obliteration of the pericardial sac and occasional adhesion to neighboring extracardiac structures; 4 of them had a serofibrinous or serohemorrhagic exudate partially sacculated between the adhesions. Of the remaining 4 cases in Group II, 3 revealed localized fibrinous and fibrous lesions over the auricles or auricular appendages, and only 1 had a grossly normal pericardium.

In Group III (recurrent attacks of acute rheumatic fever) all of the 12 cases showed some evidence of gross pericarditis. In 7 in which it was universal, 6 had complete obliteration of the pericardial cavity, and the 7th, the youngest patient in the group (4½ years), had a serous effusion with fibrinous exudate and easily separated adhesions. In the remaining 5 cases the lesions were localized in isolated areas of the pericardium.

Group IV, comprising 28 cases where death had apparently occurred of cardiac decompensation but where myocardial Aschoff bodies were present on histological examination, included 4 cases with gross universal pericarditis and 6 with local pericarditis in the form of isolated adhesions or patches of thickened pericardium. The remaining 18 were grossly normal. In 3 of the cases with universal pericarditis the lesions consisted of fibrous adhesions with thickening (in 1 there was a localized calcareous mass in the pericardium), and in the 4th there was a serosanguineous exudate with fibrinous deposits and adhesions which could be easily separated. Two of the cases with localized pericarditis likewise showed a serofibrinous exudate.

In Group V, comprising 19 cases with chronic, rheumatic valvular disease but without Aschoff bodies in the myocardium, 4 had a universal pericarditis consisting of an adherent pericardium with obliteration of the pericardial sac, 5 showed localized fibrous or fibrinous pericardial thickenings and 10 were grossly normal.

The purest form of acute pericardial lesions can be studied best in Group I where there was but one attack of rheumatic infection. Although the attack was severe enough to end fatally in these cases, the

local pericardial infection was either too mild or of too brief duration to give gross lesions in 3 of the 10 cases, and caused only local lesions in a 4th case. In the other 6 cases with generalized pericardial inflammation, a fibrinous pericarditis was visible, and despite the brief duration of the process (as early as 3½ weeks in some cases), there was already distinct evidence of healing in the form of grossly visible adhesions. Furthermore, half of these 6 cases presented a significant pericardial effusion.

In Groups II and III, in which there was more than one attack of rheumatic fever and where death occurred during an acute exacerbation, only 1 case appeared normal on macroscopic examination. These groups, in contrast to Group I, presented a more advanced state of healing with scar formation. In half of the cases adhesion between the pericardial layers was so extensive as to completely obliterate the pericardial cavity. In the remaining cases the pericardium was thicker than in Group I, and the fibrinous exudate much more extensive and showed more advanced organization.

The infrequency of generalized pericardial involvement in Groups IV and V can be correlated with the absence or mildness of clinical rheumatic manifestations in these cases. In spite of the fact that there was definite pathological evidence that such rheumatic infection had not only occurred in the past but, as in Group IV, had been active at the time of death, the customary exudative phenomena in the serous membranes (pericardium, synovia, and so on) may have been extremely mild.

In all five groups when a localized pericarditis was present there seemed to be a tendency for this to occur more frequently over the posterior wall of the left auricle. This area, therefore, should be carefully examined for such lesions. These may be rather insignificant and easily overlooked, particularly as they are apt to form inconspicuous adhesions at the cephalad portion of the pericardial fold. It is possible that this left auricular localization of the pericardial process may represent a contiguity spread of an auricular endocardial lesion.

This site of predilection for localized lesions and sacculated effusions, together with the occasional presence of an isolated fibrous tag on the posterior wall of the ventricular chambers near the apical region, was pointed out to the authors by Dr. Emanuel Libman.[27]

Histology of the Normal Visceral Pericardium

The visceral pericardium (Fig. 1) is composed of three layers: superficially, the epithelial layer; below this, and frequently separated from it by a loose reticular zone of varying thickness, is the fibrous or fibroelastic lamina propria; between the latter and the myocardium there is an adipose layer containing numerous nerves, blood vessels and lymphatics. The epithelial layer consists of a single row of mesothelial cells, generally not as flat as those lining other serous membranes, but varying in shape according to their location and the phase of the cardiac cycle. Usually they are cuboidal, their margins quite distinct, the protoplasm finely granular and the nuclei oval or round and staining deeply. In routine sections these cells are not always clearly visible, but the surface of the pericardium appears bound by a fine line (membrane) with occasional flat nuclei, in distinction to the raw appearance resulting when these cells have been desquamated by injury.

The structure of the lamina propria, a rather loose fibroelastic layer of variable thickness, is somewhat dependent on the age of the individual but its variations are not as marked as in other portions of the heart, such as blood vessels, auricle, and so on. At 3 months it is quite slender and consists of a framework of loose interlacing areolar tissue containing small irregular whorls of dense collagen. There are also present comparatively few, loosely arranged, fine, wavy elastic fibers. These are more conspicuous where elastic reinforcements are received from the moderately elastified adventitia of the pericardial vessels. At this early age the lamina propria is not yet definitely separated from the other two layers as there is comparatively little adipose tissue and the collagenous tissue is intermingled with the fat cells.

A clearer distinction into the three layers is visible in the second year. At this time the fibroelastic structure of the lamina propria appears thicker and more clearly demarcated, sometimes separated from the epithelium by an acellular loose zone of reticular tissue, and from the myocardium by a rather thick layer of adipose tissue. With increasing age the fine, wavy elastic fibers of the lamina propria become much more numerous and tend to form a distinct band on its superficial aspects parallel to the surface. This band is connected by numerous short oblique and perpendicular elastic fibers with sub-

jacent, interlacing collagenous fibers. When the loose reticular zone separating the lamina propria from the epithelial surface becomes appreciably widened, a fine elastic membrane may sometimes be found immediately beneath the latter. At times, the loose reticular zone may contain smooth muscle fibers. From the fourth decade on, the elastic fibers of the dense collagenous lamina propria are thicker and often form a rather conspicuous, regularly arranged layer of elastic tissue superficial to the collagenous mass. The latter is irregularly permeated with scattered elastic fibers.

The adipose layer consists of the classical signet ring cells with wavy, circular or oval outline. A fine reticular framework running through this tissue contains occasional arterioles and delicate capillaries at the junction of fatty lobules. There are also present numerous larger vessels in the region of which the ordinarily delicate stroma becomes strengthened by offshoots from the vascular adventitia. Where the adipose layer adjoins the lamina propria there are numerous small vessels which only rarely extend into the latter. Numerous nerves are found, especially in the region of the blood vessels.

Histiocytes were generally present and leukocytes were found occasionally in the various pericardial layers of the hearts that constituted the normal group. The histiocytes appeared in moderate numbers in the lamina propria and more sparsely in the adipose layer. Leukocytes, when present, were invariably of the mononuclear variety, usually small lymphocytes. They were rarely seen in the lamina propria and then only in small numbers. In the adipose layer their presence was somewhat less rare. They were usually observed in small numbers in the vicinity of small blood vessels. These cells probably represent evidences of pericardial irritation occurring either in the course of non-rheumatic infections present in these cases before death, or due to mechanical irritation (rubbing) of an enlarged auricle or ventricle. These cellular collections may bear some resemblance to the mild lesions in Groups IV and V. In the latter, however, the cellular aggregates are more marked and more extensive and associated with inflammatory changes (increased and dilated blood vessels, and so on).

To summarize, the significant features in the normal pericardial structure are the paucity of leukocytes; the regular arrangement of the three layers; the intact epithelium with its single row of cells; the

slender layer of elastica and collagen containing but few fixed connective tissue cells and, rarely, occasional capillaries; and the adipose layer with its fatty cells, blood vessels, nerves and fine reticular stroma.

Microscopic Features of Rheumatic Pericarditis

Epithelial Alterations

One of the early changes in rheumatic infection of the pericardium involves the lining mesothelial cells which begin to proliferate, become loosened from the underlying structures and are desquamated into the pericardial cavity (Fig. 2). This may be associated with a thickening of the entire layer superficial to the epithelial basement membrane. In many cases the epithelial cells retain their configuration and may be seen lying in rows detached from the remainder of the pericardial membrane. Within a given section of the same case they may be observed in all the various stages mentioned. Particularly where gross pericarditis is absent or largely confined to localized areas, one may observe stretches of intact epithelium occasionally interrupted by sites of uncovered pericardium, or by proliferated and desquamated epithelial cells.

The frequent tendency of the desquamated epithelial cells to preserve their intact cellular structure and become detached in single-layered strips sometimes leads to bizarre pseudoglandular formations (Fig. 3). Several such strips seem to join at their free ends to enclose clear cyst-like spaces which are sometimes already filled with a serous or hemorrhagic fluid. If the fluid portion is absorbed, as in the cases reported by Lauche [21] and Bohrod,[22] solid as well as cystic tumors result, which were not, however, observed grossly in our cases. Occasionally, small polypoid structures are formed by epithelial cells which proliferate without detaching.

Usually, when there is an extensive fibrinous exudate, the epithelium is completely missing except for occasional groups of cells found free in the pericardial exudate. However, sometimes rows of intact cells are seen somewhat below the surface of the thickened epicardium, covered by an organizing fibrin-containing layer of tissue rich in cells and blood vessels. In such cases it appears as if the fibrinous material was exuded between the epithelial cells without disturbing them. Not only fibrinous exudation but certain other

alterations in the subjacent collagen, in the blood vessels or in the fatty layer, will be described as occurring occasionally while the epithelium is still intact. From these and other observations to be mentioned, it appears that while the changes in the pericardial epithelium occur early, they are neither the initial nor the essential feature of rheumatic pericarditis.

Changes in the Lamina Propria

It is generally difficult to determine the earliest stages in the inflammatory process, so obscured are they by the extensive microscopic alterations in the pericardium which have already occurred even when death has supervened comparatively early in the course of a first attack of rheumatic fever. But in the occasional sections in which the earlier and milder lesions can be observed free from later secondary complication, the lamina propria and, more specifically, its collagen undergo the same swelling and degeneration that characterize primary rheumatic lesions elsewhere in the heart. The normally regular, interlacing feltwork of collagen is seen to be swollen, edematous, irregularly frayed and distorted. The entire lamina propria, particularly the portion adjoining the adipose layer and containing the greatest concentration of collagen, becomes infiltrated with numerous inflammatory cells, all or most of which are round cells (Fig. 4). The majority of these are small with scanty cytoplasm and deeply staining nuclei resembling or identical with the lymphocyte, but there are also numerous large round cells with considerable cytoplasm and oval or round vesicular nucleus, and varying numbers of plasma cells. Occasionally, foci of polymorphonuclear leukocytes appear which do not seem to be regular constituents of the rheumatic infiltrate.

In some of these early lesions the degenerated collagen takes on eosinophilic properties beneath an epithelium still intact except possibly for some proliferation and beginning desquamation. At this stage there may still be no fibrinous exudation, but there is usually an increased vascularization especially at the junction of the adipose layer with the lamina propria but also within the latter layer itself. Frequently, the smaller arterioles, venules and capillaries show swelling and proliferation of the endothelium, a phenomenon often characterizing rheumatic vascular lesions elsewhere. When

fibrinous exudation occurs, organization soon follows and the lamina propria can be seen clearly demarcated by a line of elastic tissue from the organizing exudate (Fig. 5).

In some cases, particularly in Group I, conspicuous lesions were seen within the pericardial wedge adjoining the ring of the mitral and tricuspid valves and in the deeper portion of the adipose layer near the myocardium (Fig. 6). These lesions consisted of edema, excessive vascularization, infiltrations with round cells, polymorphonuclear leukocytes, Aschoff bodies and the tendency to early and marked fibrosis. Comparison of these lesions with those in the adjoining ring or subjacent myocardium suggested that the pericardial alterations were due to extension of the inflammatory process from the first mentioned structures. Such pathogenesis, however, did not exclude a simultaneous involvement of the pericardium in its more superficial aspect, as already described, probably through the blood vessels adjoining the lamina propria.

Changes in the Adipose Layer

Very early in the course of the inflammatory process the adipose layer shows pathological alterations which, when well marked, indicate a full blown process involving every portion of the pericardium. The earliest changes appear to be an edema, cellular infiltration and increased vascularization of this layer. Sometimes there is extravasation of red blood cells into the fatty tissues or superficially into the pericardial cavity. The fine reticular stroma forming the framework becomes thickened, irregular and greatly increased in amount. The normal outline of the fat cells becomes distorted and compressed by the surrounding edema and exudate. The inflammatory cells resemble those already described except that while round cells still predominate, there is more often a considerable representation of polymorphonuclear leukocytes. The cellular infiltration tends to form foci around the smaller branches of the pericardial blood vessels.

Advanced Pericardial Changes

The well developed case of rheumatic pericarditis presents a markedly thickened pericardial membrane covered by a dense layer of fibrin containing a few epithelial and inflammatory cells. Below lie the remains of the lamina propria, swollen, edematous, distorted and

sometimes still showing eosinophilic swelling and degeneration. In this area there is an intense inflammatory exudate consisting chiefly of round cells and occasionally of polymorphonuclear leukocytes and nuclear débris. In addition there is marked vascularization of this region which contains numerous small arterioles and capillaries, many of which show endothelial proliferation, swelling and other vascular changes to be described. There is marked tendency for the inflammatory cells to concentrate around these blood vessels. Occasionally, at this stage, an Aschoff body is present. When organization is already visible, as is usually the case, there are in addition numerous fibroblasts and congested capillaries of the characteristic granulation tissue type.

In the adipose layer the normal regular conformation of the fat cells has become obliterated by an albuminous and sometimes fibrinous exudate. There is an increase in the reticular stroma which often forms thick bands running through this layer. Throughout there is an intense vascularization and leukocytic infiltration, especially around the blood vessels. The formation of granulation tissue appears to occur particularly from this layer. At an early stage there is an unusual preponderance of capillaries and arterioles congested with erythrocytes. These vessels often have a corkscrew appearance and run perpendicularly toward the surface. There are numerous fibroblasts and, very early, a marked formation of connective tissue. Where organization is further developed, fibrosis may extend even to the surface and often the remnants of fibrin are visible as masses enclosed within this organizing granulation tissue.

In those cases where recurrent attacks of rheumatic fever occurred there is a definite formation of layers (Fig. 7). The original adipose layer shows marked fibrosis sometimes consisting of a vascular connective tissue with numerous round cells and a frequent increase of collagen. Part of the fatty tissue may still be visible, markedly infiltrated with fibroblasts, congested capillaries and inflammatory cells. Above the adipose layer lies the thickened and generally inflamed lamina propria. This is often separated by a fine distinct line of elastica from the next and most superficial layer. The latter consists of young granulation tissue showing an intense infiltration with round cells and fibroblasts. The surface of this last layer is frequently covered with fibrin already invaded by granulation tissue with irregular patches of complete organization. In a great number of cases in this

group this visceral pericardium with its partially organized fibrinous exudate is undergoing adhesion with the similarly altered parietal layer. Between the two inflamed layers of the pericardium there is an extremely loose granulation tissue consisting of fibroblasts, connective tissue fibers and granulation tissue capillaries. In these cases it is difficult to say whether the deeper fibrotic layers represent the healed inflammatory process of previous attacks or whether all the changes described have occurred within the last attack itself. The latter, if true, would indicate a greater tendency to early fibrosis in cases where previous attacks of rheumatic fever had occurred than in those where the patient succumbed in the first attack.

Lesions in Groups IV and V

Certain mild pericardial changes which differed in some respects from the early lesions described above were especially frequent in the cases in Groups IV and V in which there was no history of rheumatic fever. The essential feature in these cases was the presence of inflammatory cells and blood vessels in the deeper portion of the lamina propria adjoining the adipose layer (Fig. 8). Normally, as we have indicated, the lamina propria is invariably or almost invariably free from blood vessels or cells except for occasional fixed connective tissue cells. In 38 of the 47 cases constituting Groups IV and V, this layer showed infiltrations with small and large round cells largely resembling the inflammatory cells described in the more acute cases of pericarditis. They were, however, less numerous and less extensive than in the acute cases already described. Furthermore, there were to be seen numerous capillaries, arterioles and small arteries, some of which showed alterations like those seen in other cases of rheumatic fever. Sometimes these inflammatory changes were present in one or two of the standard sections and not in any of the others, occasionally being found only in the pericardium which lies behind the aorta or pulmonary artery (Gross [28]).

In 4 of the cases in Group IV, Aschoff bodies were found in the pericardium, in 1 case in the immediate vicinity of a large coronary artery, and in 1 case associated with a banded arrangement of small and large round cells. While the most superficial layer of the pericardium was at times thickened, there were no reduplications such as are seen in old rheumatic auricular lesions. However, a definite

disorganization of the collagenous and elastic structure of the pericardium was often seen. In the adipose layer there was an increased amount of stroma and numerous localized foci of round cells, especially around the generally more numerous blood vessels. In not all of the cases in Groups IV and V were the changes limited to those just mentioned. An occasional case revealed fibrinous exudation. The surface epithelium was usually intact, but there were occasional cases showing epithelial proliferation, pseudogland formations or polypi on the surface. In addition to the above findings some cases showed fibrosis and adhesions, occasionally with increased elastification of the pericardium. In a large majority of cases there was not merely an increase in the number of blood vessels throughout the pericardium but, in addition, other rather characteristic qualitative vascular alterations such as described by Gross, Kugel and Epstein [29] for other portions of the heart.

Alterations of Blood Vessels

Most of the blood vessels seen in acute pericarditis are dilated congested capillaries, often round but more characteristically elongated, tortuous, or of corkscrew shape with the long axis perpendicular to the surface. These were generally components of granulation tissue in the course of healing. Certain other capillaries, apart from granulation tissue, showed in their endothelial linings a striking swelling and proliferation which was occasionally sufficient to form a verrucous projection into the lumen. Rarely, the proliferated endothelial cells became desquamated and degenerated to form a plug which became organized and recanalized. Not infrequently in the active cases small pericardial veins contained thrombi. The small arteries and arterioles in the majority of cases showed changes identical with those described by Gross, Kugel and Epstein.[29] Frequently there was a marked increase of fibrous tissue in the adventitial and periadventitial region of the blood vessels. In the cases of the first three clinical groups there was not infrequently medial hypertrophy, especially behind the aorta and in the wedge of pericardium near the auriculoventricular junction, and sometimes intimal fibrosis occasionally with increased elastification of the intima. Perhaps the most frequent and characteristic lesion was that which Gross, Kugel and Epstein [29] have termed the intimal musculo-elastic hyperplastic

lesion, a marked development of longitudinally arranged smooth muscle cells which constitute several layers internal to the lamella elastica interna, and an increased elastification of the intima, sometimes with swelling of the endothelial cells. Because of their distinctive appearance and frequent occurrence in rheumatic fever, these lesions, which were found not infrequently in the cases of Groups IV and V, as well as in the more active cases, formed important corroborative evidence of rheumatic infection of the pericardium. Intimal musculo-elastic hyperplastic lesions were especially numerous in the pericardium behind the pulmonary artery and aorta.

Aschoff Bodies

Aschoff bodies apparently identical with those observed in the myocardium, were found in 2 cases of Group II, in 1 case of Group III, in 4 cases of Group IV, but with the greatest frequency in Group I where they were present in 6 of the 10 cases. They were thus present in about one-fifth of the cases in which Aschoff bodies were found elsewhere in the heart. Observed in the pericardium most often in relation to branches of the coronary arteries, occasionally they occurred independently of adjacent blood vessels and sometimes could only be found in the pericardium behind the pulmonary artery or aorta. In the free connective tissue they generally assumed the reticular or mosaic form described by Gross and Ehrlich,[24] but in the proximity of blood vessels, especially of larger size, there was a greater tendency toward the polarized form (Fig. 9).

Discussion

Incidence of Pericarditis in Rheumatic Infection

A detailed, macroscopic and microscopic study of the pericardium in rheumatic heart disease made by Coombs [30] revealed a progressive diminution of pericardial lesions varying from 100 per cent in patients dying in the first decade to about 25 per cent in those dying in the fourth or subsequent decades, with a total mortality of 53 per cent. According to Coombs the lesser incidence in the later age periods indicated that these patients suffered from milder lesions and therefore survived longer. The average incidence of gross universal pericarditis in the first three groups was 70 per cent, with local pericardial lesions in an additional 20 per cent. In Groups IV and V

there was an average of 17 per cent showing universal pericarditis with local pericarditis in an additional 23 per cent of the cases. Microscopically, however, there was distinct evidence in one or more of the sections of pericardial inflammation in 100 per cent of the cases in each of the first three groups, in 85 per cent of the fourth and in 75 per cent of the fifth. These figures on the incidence of gross and microscopic pericarditis are somewhat higher than those reported by Gross [31] in his study on the left auricular lesion in rheumatic fever.

Correlation of Pathological Findings with Clinical Groups

The microscopic appearance of the various pericardial lesions can be segregated into three general patterns. The first is represented most clearly in Group I and is characterized by early acute lesions found only in the cases in which there was a single attack of rheumatic fever. These lesions consisted primarily of swelling and degeneration of collagen found chiefly in the lamina propria, and inflammation and vascularization of the same layer and nearby portions of the subjacent layer. In addition there was an exudation of fibrin which was the more conspicuous when the above mentioned preliminary changes were extensive. The overlying mesothelium was intact or showed mild proliferative changes in the less severe cases and complete desquamation, sometimes with the formation of pseudogland structures or polypi, in the more severe cases. Aschoff bodies were found with the greatest frequency in this group. Adhesion between the layers of the pericardium occasionally occurred but this was not a frequent or a characteristic finding.

Groups II and III, consisting of cases of recurrent rheumatic fever, formed in many instances a second histological pattern. The essential lesions in these cases were characterized by the marked thickening of the pericardial membranes, by an almost universal formation of adhesions with the tendency to obliteration of the pericardial cavity, by well marked organization of the inflammatory exudate, by the formation of layers and by the presence of remains of the inflammatory changes such as noted in Group I.

The third histological pattern was found in Groups IV and V. These were mild lesions characterized by the infiltration of round cells into the deeper portions of the lamina propria and adjacent

areas, by increased vascularization and structural alterations of the blood vessels. There was an absence of fibrinous exudation or other evidence of acute inflammation. The overlying epithelium in general was intact and there was no formation of adhesions.

The earliest and mildest pericardial lesions in rheumatic fever probably occur in those patients who suffer a single attack of the disease without succumbing. Such cases could be obtained only if immediately following recovery from rheumatic fever the patient succumbed to some unrelated accident. We may surmise that the lesions in these cases would not differ essentially from the early mild lesions found in occasional sections in Group I. The other lesions that have been described for Group I represent the course of the rheumatic infection when this has been sufficiently severe to cause death. On the other hand, it is possible that the mild lesions that were described for Groups IV and V represent the course of the rheumatic infection when the patient survives. In other words, these lesions may be the residual findings from a preceding rheumatic pericarditis of a mild nature. It is difficult to say whether in these cases there once was an acute, exudative fibrinous pericarditis with loss of epithelial lining and with subsequent restoration to complete integrity, or whether the original lesions were so benign that there never was a generalized irritative reaction on the part of the lining membrane. Correlating this with the absence of clinical features of rheumatic fever, one might then assume that in these cases there was a similar absence of exudative phenomena elsewhere in the body (for example, joints) which usually determines the recognition of the clinical picture.

The type of lesion generally found in cases of recurrent rheumatic fever we believe represents the course of a severe pericardial infection with acute exudative changes which, although not resulting fatally, were nevertheless too extensive to permit any complete restitution. In these cases such healing as occurred did not result in a completely normal structure but led to pericardial thickenings, adhesions and obliterations which in themselves were undoubtedly an unfavorable contributing influence on the course of the disease.

With this interpretation of the various pericardial pictures it would seem that almost in 100 per cent of the cases of rheumatic disease of the heart the pericardium was affected with certain inflammatory changes. However, only in a variable percentage of such

cases are these lesions severe enough to lead to the striking exudative membrane reactions that characterize the obvious pathological and clinical manifestations by which we recognize the acute rheumatic infection. For this reason, in chronic rheumatic cases, such as those found in Groups IV and V, there is no clinical history of rheumatic fever and little or none of the characteristic gross pathological appearances of that disease in the pericardium. Only careful microscopic examination reveals that even in these cases the pericardium has not been immune from rheumatic involvement.

Summary

There have been described in this report the gross and microscopic pericardial lesions found in 87 cases of active and inactive rheumatic fever. These cases were divided into five clinical groups depending on the course of the disease. It is shown that while each group may present individual differences, the lesions as a whole fall into three more or less characteristic histological patterns. Data on the incidence of the various lesions are presented in which it appears that rheumatic disease of the heart is almost invariably associated with certain inflammatory changes in the pericardium. A description is given of the age period changes in the histology of the normal pericardium.

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DESCRIPTION OF PLATES

PLATE 21

FIG. 1. Structure of normal pericardium. Age 6½ years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = lamina propria. Note loose tissue separating this layer from overlying epithelial layer bound by delicate elastic membrane; B = adipose layer; C = myocardium.

FIG. 2. Superficial layers of visceral pericardium from active case of rheumatic fever. Age 19 years. Medium power. Hematoxylin and eosin stain.

A = proliferating epithelium; B = denuded area. Note beginning separation of epithelial layer from underlying tissue. C = loose tissue between epithelial layer and lamina propria showing edema and containing lymphocytes, polymorphonuclear leukocytes and congested capillaries; D = lamina propria showing swelling and eosinophilic change.

FIG. 3. Visceral pericardium from active case of rheumatic fever showing polypoid and pseudogland formations. Age 39 years. Medium power. Hematoxylin and eosin stain. Note proliferating cuboidal epithelium and hemorrhage beneath the epithelial layer.

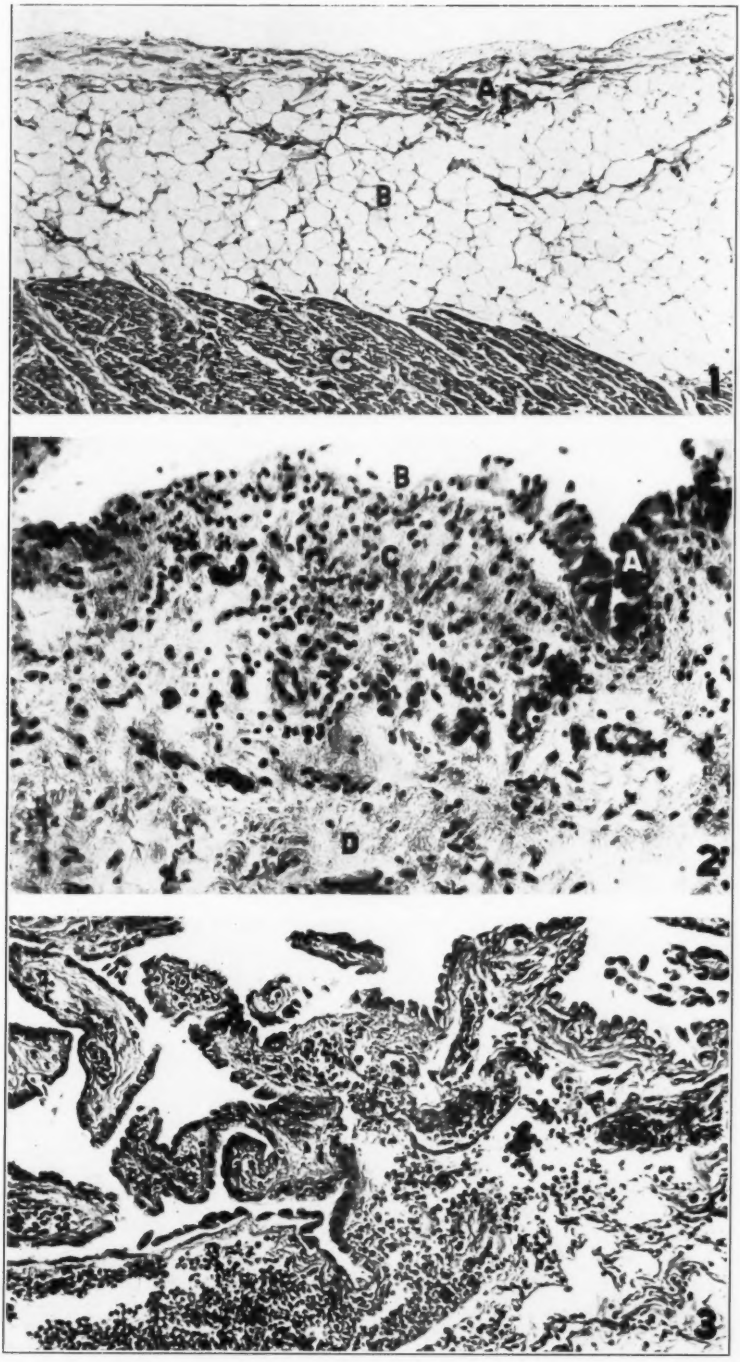

A
B
C
1
B
C
A
D
2
3

PLATE 22

FIG. 4. Visceral pericardium from active case of rheumatic fever. Age 17 months. Low power. Hematoxylin and eosin stain.

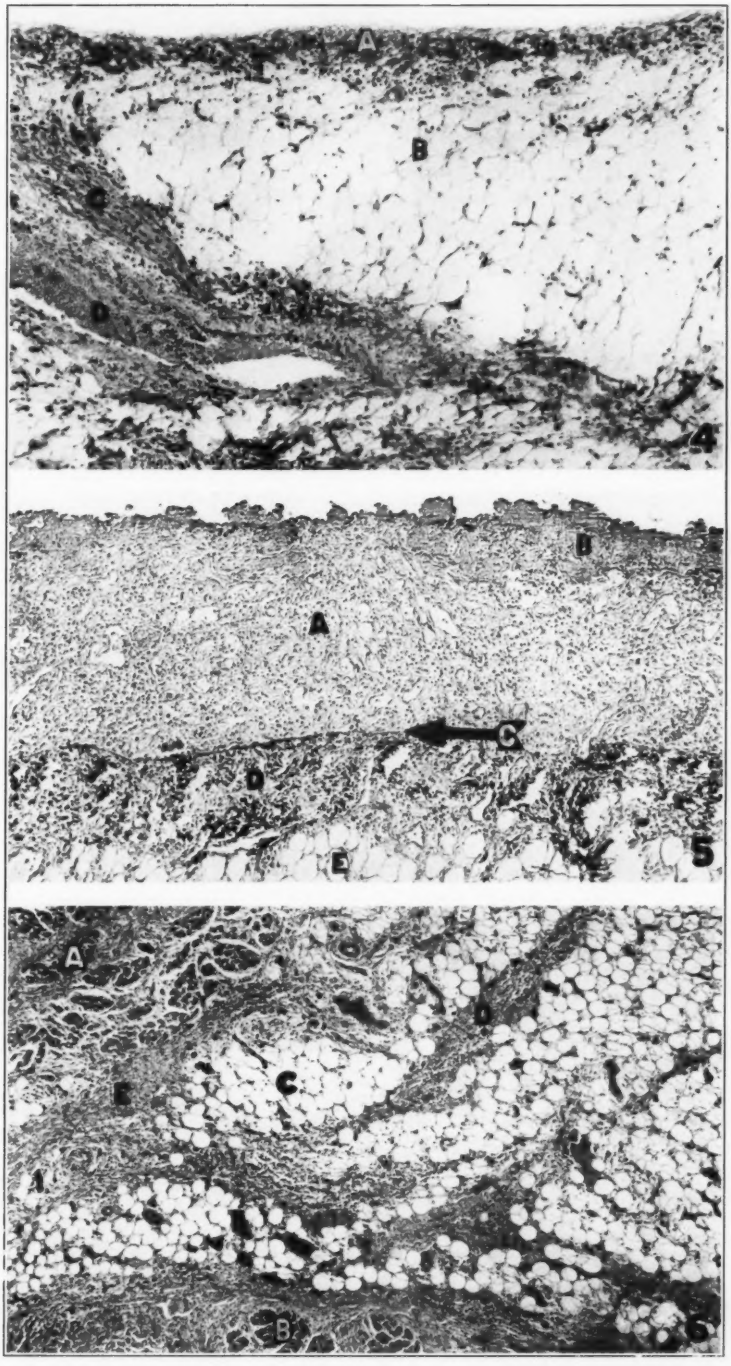
A = lamina propria infiltrated with leukocytes and showing congested vessels in vicinity; B = moderately inflamed adipose layer; C = strip of myocardium; D = large coronary branch in adipose layer. Note inflammatory cell infiltration in lower zone of adipose layer.

FIG. 5. Superficial layers of visceral pericardium from active case of rheumatic fever. Age 13½ years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = organizing granulation tissue which has almost completely replaced exudate on surface of visceral pericardium; B = remains of unorganized fibrin; C = elastic lamella separating lamina propria from organizing exudate; D = considerably inflamed lamina propria showing marked infiltration and dilated blood vessels; E = adipose layer beneath the lamina propria showing thick fibrous septa between fat cells and lobules.

FIG. 6. Pericardial wedge between left auricular and ventricular myocardium. Age 20 years. Low power. Hematoxylin and eosin stain.

A = left auricle; B = crest of posterior wall of left ventricle; C = adipose tissue constituting the pericardial wedge; D = aggregation of inflammatory cells and fibrin; E = connective tissue replacement of exudate.



Friedberg and Gross Pericardial Lesions in Rheumatic Fever

PLATE 23

FIG. 7. Visceral pericardium from active case of rheumatic fever. Age 20 years. Very low power. Weigert's elastic and Van Gieson's connective tissue stain.

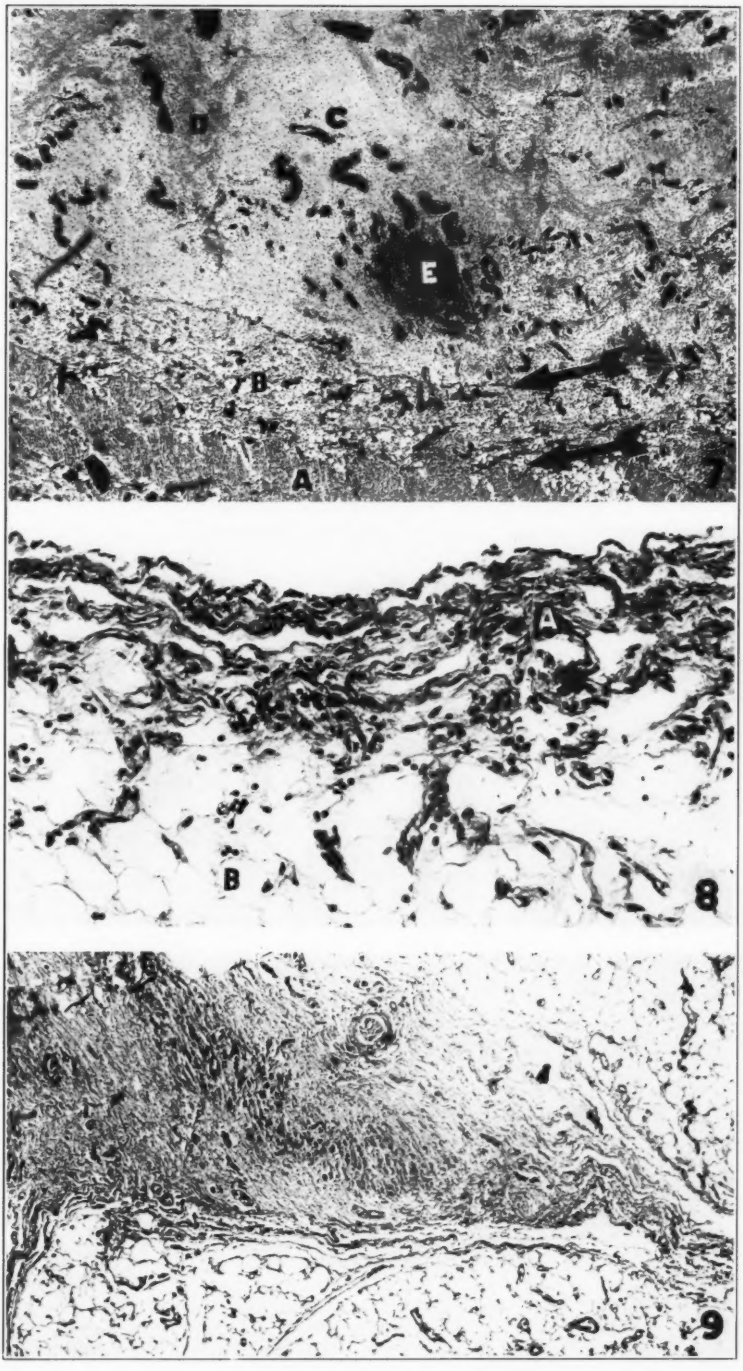
A = myocardium; B = visceral pericardium which has been almost entirely replaced by fibrous connective tissue. Note relatively few remaining fat cells, inflammatory cell infiltration and marked vascularization. Some of the blood vessels are seen to penetrate the upper limiting elastic lamella lying above the lamina propria (upper arrow). The lower arrow marks the level of the ventricular myocardial crest. C = edematous organizing and organized exudate superficial to the visceral pericardium; D = unorganized fibrin; E = hemorrhage.

FIG. 8. Superficial layers of visceral pericardium from inactive case of rheumatic fever. Age 42 years. Medium power. Hematoxylin and eosin stain.

A = lamina propria showing infiltration with lymphocytes. Note numerous engorged capillaries within the lamina propria and immediately below it; B = moderately inflamed adipose layer.

FIG. 9. Visceral pericardium from active case of rheumatic fever. Age 27 years. Low power. Hematoxylin and eosin stain.

Conglomerate Aschoff bodies (polarized type) embedded in adipose layer of visceral pericardium.


D
C
E
B
A
A
B
8
9

SUBCLINICAL ADENOMA OF THE PITUITARY GLAND *

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INTRODUCTION

For many years the pituitary gland, or hypophysis cerebri, has been one of the favorite subjects for scientific investigation. In the early years of the present century interest in it, as indeed in most other organs, centered on its anatomical and pathological aspects. As physiological methods and knowledge improved, more and more attention was paid to its physiology, and in the past few years probably the chief interest has been in the latter field, particularly with reference to its effect on the other glands or organs of the endocrine system.

During this interval, however, and in spite of predominant interest in the physiology of the gland, several men, including Dandy and Rasmussen, investigating its anatomy, and Cushing and Bailey its pathology, have advanced our knowledge materially in these fields. The publication by Cushing [1, 2] in 1932 of a series of cases describing a clinical syndrome often associated with basophilic adenoma reawakened general interest in some of the pathological manifestations of the pituitary gland. This renewed interest centered particularly around those long neglected and minute adenomas which occur in the anterior part of the pituitary gland and which I propose to call subclinical adenomas. Up to the present these adenomas have been considered chiefly as pathological curiosities.

Adenomas of microscopic size or larger are found to occur rather frequently in the glands of the endocrine system. Adenomas in the thyroid gland are so frequent as to be almost normal findings. Adenomas of microscopic size or larger are found in at least 25 per cent of all suprarenal glands examined at autopsy. The kidney, while not an endocrine organ, may be the site of numerous tiny adenomas in

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both the cortex and medulla and these adenomas may not produce any clinical symptoms. The prostate gland, again not an endocrine organ but one perhaps with some endocrine functions, shows a marked tendency to adenomatous formation with advancing age. For this reason it would be reasonable to suppose that symptomless adenomas in the pituitary gland should not be particularly uncommon.

It was not the purpose of this investigation to attempt to refute claims as to the clinical significance or insignificance of these adenomas. All of those in the present series were found in the pituitary glands of subjects who, at least so far as can be determined, had died of causes unrelated to disease in that gland. The study was undertaken chiefly to determine how frequently adenomas occur in supposedly normal pituitary glands, and incidentally to learn the ratio of frequency of adenomas composed of the three types of cells that are found normally in the anterior lobe, that is, chromophobic, eosinophilic and basophilic cells.

Such adenomas in the anterior lobe of the pituitary gland have been recognized for many years. Erdheim [3] in 1903 mentioned finding 2 adenomas of minute size in the hypophysis, both of which were apparently composed of basophilic cells. Löwenstein [4] in 1907 mentioned finding 2 more, but he neglected to mention their cell type. Erdheim and Stumme [5] in 1909, writing on the changes in the anterior lobe of the pituitary in pregnancy, reported finding 10 minute adenomas in 118 cases, or in 8.4 per cent. Roussy and Clunet [6] in their paper on tumors of the anterior lobe of the pituitary in 1911 also mentioned small adenomas found at autopsy that had apparently not caused clinical symptoms.

In spite of Erdheim and Stumme's [5] report of an incidence of approximately 10 per cent, these adenomas continued to be considered very rare until 1933, when Roussy and Oberling [7] stated that adenomatous foci occurred in 10 per cent of the pituitary glands examined at autopsy; however, they gave no statistics on the number of cases. In the same year Susman [8] found 23 adenomas in 22 of 260 pituitary glands from routine postmortem examinations, a percentage again of 8.4. Of these 23 adenomas 8 were composed of basophilic cells, 6 of eosinophilic cells and 5 of chromophobic cells; the remainder were not classified. These adenomas varied in size from 0.2 to 25 mm. In 1934 Close [9] reported that the incidence of

pituitary adenomas was approximately 10 per cent in a series of routine postmortem examinations, but that it ran as high as 44 per cent in those cases in which there was associated carcinoma in other glands. Many others, such as Stolkind,[10] Wall and Hoyle,[11] Pritchard,[12] Rutishauser,[13] Weber,[14] Russell, Evans and Crooke,[15] Moehlig,[16] Bishop and Close,[17] Teel,[18] Wieth-Pedersen,[19] Kraus,[20] Reichmann,[21] Anderson,[22] Bauer and Wassing,[23] Roch,[24] Craig and Cran,[25] and Ulrich,[26] have reported 1 or more cases of basophilic adenoma associated with the syndrome known as pituitary basophilism.

Source and Preparation of Material

The material for this study consisted of 1000 pituitary glands obtained over a period of years in the course of routine postmortem examinations in which permission to examine the brain had been granted. The pituitary gland was removed in these cases by clipping off the posterior clinoid processes with bone forceps and removing the gland *in toto*. It was then placed in 10 per cent formalin solution and stored.

In preparing the sections the glands were removed from the formalin and cut into sagittal sections paralleling the longest axis. Since the glands vary greatly in shape and size, this method resulted in sections from some glands being parallel to the anteroposterior diameter, whereas others were transverse. The sections were cut by hand in thicknesses varying from 1 to 1.5 mm. Since the majority of adenomas were found to be 1.5 mm. or larger in diameter, it was felt that by this method the likelihood of many being missed on section was fairly remote. It might be mentioned at this point that attempts to identify adenomas in the freshly cut sections were in most cases futile.

These sections were then placed in Zenker's fluid for 24 hours to facilitate later staining by the Mallory-Heidenhain method. They were then embedded in paraffin in the ordinary manner and two sections, 10 microns in thickness, were cut from each paraffin block and these were mounted and stained with hematoxylin and eosin in the usual way. By this method three to ten sections from each gland were stained. The hematoxylin and eosin sections were examined grossly and microscopically, and in those cases in which adenomas were found another section was cut from the corresponding block of tissue

and stained by the Mallory-Heidenhain method, as outlined by Kernohan,[27] to determine the types of cells composing the adenoma. With this stain the cytoplasm of the chromophobic cells either does not stain at all or is pale blue; the eosinophilic granules stain bright red and the basophilic granules stain dark blue. These sections were then used to classify the adenomas as to their cell types.

Characteristics of Adenomas

At this point it becomes necessary to consider what constitutes an adenoma. The basophilic cells in particular have a tendency to occur normally in clumps or islands, which on superficial examination look like small adenomas but are probably merely normal architectural variations. Probably there can be no absolute criterion by which one can differentiate true adenoma formation and unusual structural variations. The smaller adenomas in the anterior lobe of the pituitary have no demonstrable capsule, and even in the larger ones what at first glance appears to be a capsule is merely a condensation of the cells surrounding the adenoma. In the majority of cases, however, there is no difficulty in distinguishing between an adenoma and a structural abnormality. The majority of adenomas found were spherical or nearly so (Fig. 1) and the larger ones were well demarcated by the ring of compressed pituitary cells surrounding them.

Another distinguishing factor is that in an adenoma the percentage of one particular type of cell in a given area is a great deal higher than in the normal portions of the gland. Moreover, the cell pattern differs from the normal arrangement of acini bordering vascular sinuses. In some of the adenomas the pattern resembles the honeycomb arrangement of pavement epithelium (Fig. 2); in others it looks like a markedly convoluted papilloma (Fig. 3) or compound tubular gland (Fig. 4). In still others there is no apparent structure, but the cells appear to be scattered loosely around a few vascular sinuses, with little connective tissue framework. Some also reveal areas of degeneration, necrosis and vacuole formation.

Just as the structure of the adenomas varies greatly, so do the cells show marked variability in appearance in different adenomas. In some, as in those appearing to be composed of pavement epithelium (Fig. 2), the cells are large and clear with centrally placed

and somewhat pale staining nuclei. In others the cytoplasm is somewhat scanty, the outline of the cell irregular, and the nucleus eccentrically placed. While the cells differ greatly in appearance in different adenomas, even in those made up of the same type of cells, in general they are fairly constant in a given adenoma. It was also noted that there was marked variability in the staining characteristics of different adenomas of the same type. In the eosinophilic and basophilic adenomas the color ranged from pale pink to deep red in the former and from pale lavender to deep blue in the latter. The cell types were determined by the presence of typical granules. Variations in color were not due to faulty staining technic, for the normal parts of the gland which were used as controls stained normally.

It was not found that the adenomas had any definite site of predilection for the anterior lobe, although the majority tended to be more peripherally than centrally located. Some projected from the surface of the gland under the capsule (Fig. 5), whereas others were embedded deep in the substance of the gland. Many occurred along the border contiguous to the pars intermedia, and in this region they tended to lose their spherical shape and become flattened at the intermedial pole (Fig. 6).

Adenomas were found composed of each of the three types of cell normally found in the pituitary gland. Rasmussen [28-30] has found that, in a series of pituitary glands from adult males, the anterior lobe contains on the average of 52 per cent chromophobic cells, 37 per cent eosinophilic cells and 11 per cent basophilic cells. The proportion in the glands of adult females is only slightly different from that in males. One would therefore expect that if these three types of cell were all actively growing and reproducing themselves, as some histologists believe, then in a large series of cases adenomas composed of the different types of cells should occur in about the same ratio of frequency as the cells themselves normally occur. This, however, was not found to be the case, as will be shown later.

Experience with operative specimens of pituitary tumors has shown that they are frequently composed of mixed cells. This is also true of subclinical adenomas. Not more than two or three adenomas in the series were composed of purely one type of cell; the majority, on the other hand, had one type predominating sufficiently to classify the adenoma according to this predominant type of cell. In a few

cases, however, two or even three types of cells occurred in almost equal proportions in the same adenoma, so it was necessary to classify them merely as mixed types.

In some cases more than 1 adenoma was found in a single gland. In several of these cases 2 adenomas were found to be associated, in some cases being similar types; in other cases two different types of adenoma were present. In 2 cases, 3 distinct adenomas were found, and in 1 of these cases all three types of adenoma were represented. In another case several adenomas were found, of various sizes and representing various types of cells; semiserial sections indicated that there were 10 or more separate and distinct adenomas in this one pituitary gland.

Frequency of Occurrence

Of the 1000 pituitary glands examined, 225 were found to contain 1 or more adenomas. In 224 of these glands, excluding the one with the multiple adenomas, there were found to be 265 adenomas. These adenomas were classified as follows: chromophobic, 140 (52.8 per cent); eosinophilic, 20 (7.5 per cent); basophilic, 72 (27.2 per cent); and mixed types, 33 (12.4 per cent).

Age Incidence

In an endeavor to find the age group in which adenomas most frequently occurred, the ages of those subjects with adenomas were plotted against the age curve for the 1000 subjects in the whole series. These latter had ranged from stillborn infants to a man of 99 years. The youngest subject in whom adenomas were found was 2 years old, the oldest was 86 years old.

As can readily be seen from Chart 1, the greatest incidence of adenomas occurred in the sixth decade of life; however, since the greatest amount of autopsy material also occurs in this decade, the curve in Chart 2 was plotted from the ratio of adenomas to pituitary glands in each age group. This shows even more conclusively that the highest incidence of such adenomas occurs in the sixth decade of life (Chart 2). Because of the relatively small number of subjects who were more than 70 years of age, the end of the curve is indefinite and inconclusive.

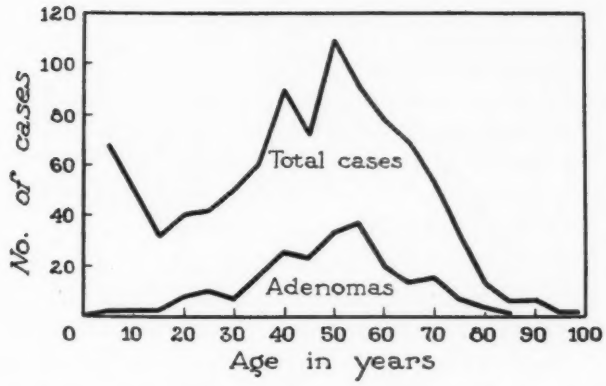

CHART 1. The ages in which adenomas occurred and the age groups in which the pituitary glands were obtained.

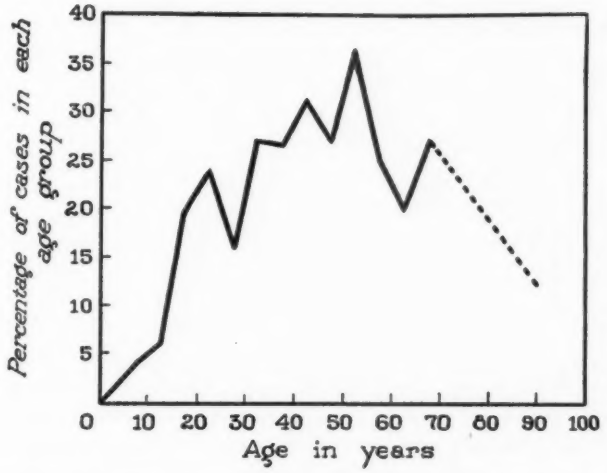

CHART 2. This graph was plotted by dividing the number of adenomas found in each age group by the total number of pituitary glands derived from subjects in that age group.

Sex Incidence

Of the 225 cases in which adenomas were found, 148, or 66 per cent, of the patients were males, 77, or 34 per cent, females. This would seem to indicate the predominance of adenomas among males. However, since the proportion of males to females in the 1000 cases was 63 to 37 per cent, it can be seen that the incidence in the two sexes is about equal.

Clinical Features

As has been mentioned before, the 1000 pituitary glands were obtained in a series of routine postmortem examinations in which permission to examine the cranial cavity had been granted. The clinical diagnoses and causes of death in these cases ranged through almost the entire list of medical and surgical conditions. As one would suspect from the age curve, diseases of middle life, such as cardiac failure, hypertension, nephritis and carcinomatosis, were more frequent than some other conditions, although the causes of death ranged all the way from stillbirth, through suicide, to senility. In the 225 cases in which adenomas were found the same variability in clinical findings and causes of death were apparent. The most notable thing about them was that there was nothing in the history or clinical findings in any case in which an adenoma was found to suggest the presence of any pituitary dysfunction. This was true in cases in which basophilic or eosinophilic adenomas were present as well as in those in which the adenomas were chromophobic. This is somewhat startling since in a few cases the adenomas were so large as almost to destroy the gland. Apparently, however, there was sufficient normal tissue remaining to sustain normal pituitary function.

Proportion of Different Types of Adenoma

As can be seen from the percentages of the different types of adenoma, the chromophobic adenomas occurred in about the same proportion as the chromophobic cells themselves occur in normal glands. With the eosinophilic and basophilic adenomas, however, there is considerable discrepancy. Although eosinophilic cells constitute 37 per cent of the normal hypophyseal cells, the eosinophilic adenomas represent only 7.5 per cent of the total number of adenomas. On the other hand, although the basophilic cells comprise only

11 per cent of the average normal cell content of the anterior lobe, basophilic adenomas made up 27 per cent of the total group of adenomas. It might be imagined that if the eosinophilic adenomas are more active than the other types, they might grow faster and give clinical symptoms early, since the majority of clinically recognized chromophilic adenomas occur before the age of 50 years. However, in the group of adenomas reported here the average age was greater than 50 years, and so this explanation is not warranted.

Of the 33 mixed types of adenomas, 21 were composed of mixed basophilic and eosinophilic cells; the remaining 12 consisted of chromophobic and basophilic cells with very few eosinophils.

Summary and Conclusions

It has been shown, therefore, that small adenomas of the anterior lobe of the pituitary gland, instead of being relatively rare pathological curiosities, occur with considerable frequency, since they occurred in 22.5 per cent of a series of 1000 unselected cases, or in nearly 1 out of every 4. Thus, the anterior hypophysis is shown to act much like other glands of the endocrine system in its tendency toward formation of adenomas.

It has been shown further that, like adenomas in other organs of the body, adenomas of the anterior lobe of the pituitary gland occur with increasing frequency in the higher age groups, and that they also apparently occur in the same proportion in both sexes.

It has also been shown that the majority of adenomas of the anterior lobe of the pituitary, irrespective of type, are entirely benign and give no recognizable clinical symptoms, and that they can be demonstrated only by rather detailed examination of the gland at autopsy. This would justify the term "subclinical adenoma" as applied to them. It is entirely possible, however, that under the influence of some unknown stimulus some of these adenomas may secrete a hormone or similar substance capable of producing clinical symptoms.

The chromophobic adenomas have been seen to occur in about the same relative proportion in the series as do the chromophobic cells themselves in the average normal gland. However, the eosinophilic and basophilic adenomas do not occur in the same relative proportions as their respective cells do in the average normal gland, and an adequate explanation for this has not been found.

Future work is yet to be done on the group of basophilic adenomas in this series in an attempt to tabulate and correlate their clinical features.

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DESCRIPTION OF PLATE

PLATE 24

FIG. 1. Chromophobe adenoma. The tendency to spherical shape, as well as the condensation of cells around the periphery, forming a pseudocapsule, is evident. Hematoxylin and eosin stain. × 23.

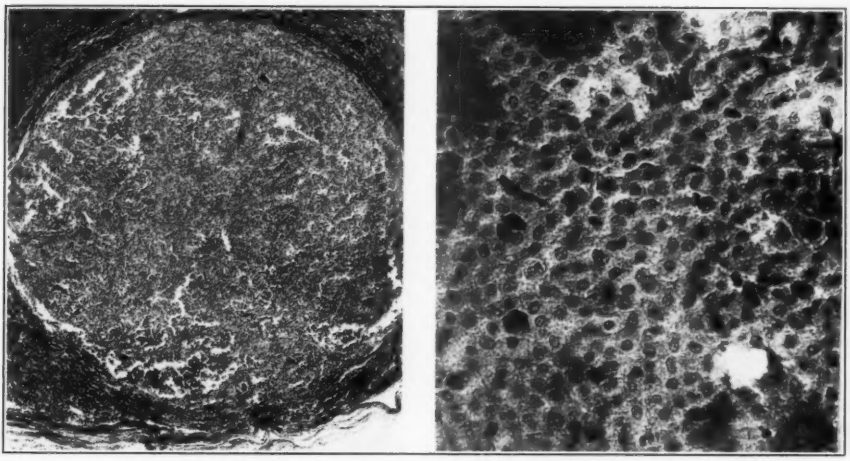
FIG. 2. Chromophobe adenoma. The adenoma cells arranged like pavement epithelium. Mallory-Heidenhain stain. × 300.

FIG. 3. Basophilic adenoma. The arrangement of cells resembles that of a convoluted papilloma. Mallory-Heidenhain stain. × 48.

FIG. 4. Chromophobe adenoma. The resemblance to a compound tubular gland is evident. Mallory-Heidenhain stain. × 85.

FIG. 5. Chromophobe adenoma. The adenoma budges the capsule of the pituitary gland. Hematoxylin and eosin stain. × 18.

FIG. 6. Eosinophilic adenoma. The adenoma is triangular and one side is against the pars intermedia of the pituitary gland. Hematoxylin and eosin stain. × 60.



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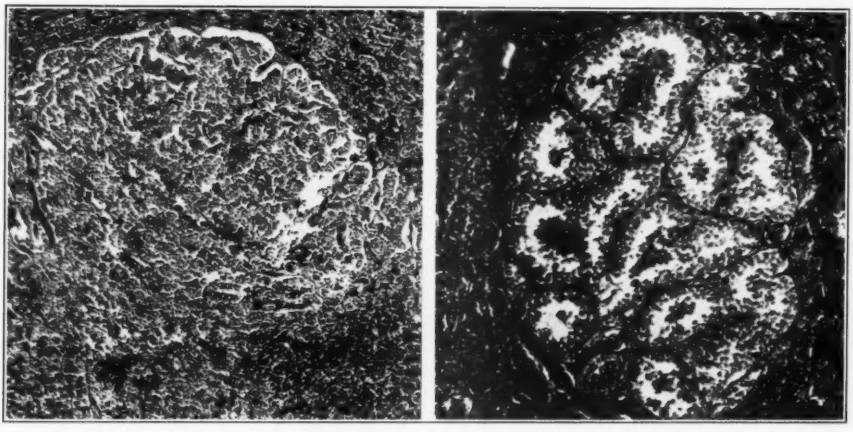

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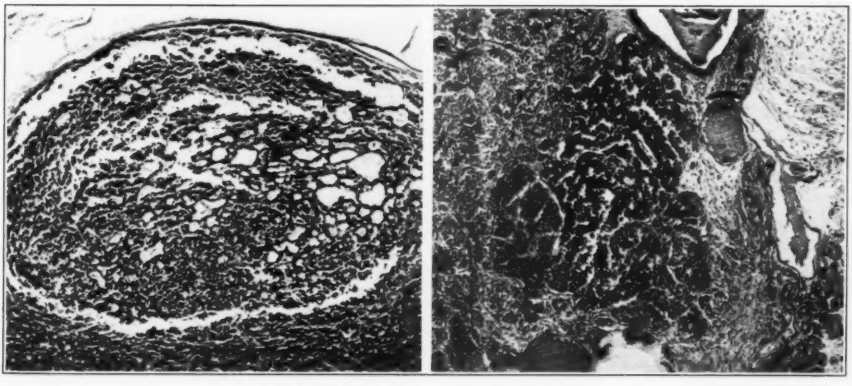

5 6

NUCLEAR ALTERATIONS FOLLOWING INTRAVENOUS INJECTIONS OF GLUCOSE AND OF OTHER SOLUTIONS *

JACK LEE

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In the hope of learning more about intranuclear inclusions in virus diseases, workers in this laboratory have proceeded along several different lines. Employing the Feulgen reaction and the Macallum test for masked iron, it has been found that the inclusions contain little or no thymonucleic acid and iron.[1] By methods of microincineration it has been shown that they consist of material that can be completely burnt away without leaving a noticeable mineral residue.†[2, 3] With the aid of the ultracentrifuge[4] the specific gravity of the inclusion material has been found to be less than that of basophilic chromatin and clear nuclear sap. Inclusion-like bodies have been reported in various experiments in which viruses are apparently not involved.[5, 6]

The purpose of the experiments herein recorded is to determine how closely experimental inclusions can be made to resemble those caused by viruses.

The literature should be considered briefly. Some investigators, in reporting experiments not primarily concerned with virus diseases, have described nuclear alterations so like those caused by viruses as to call for comment; while others have made experiments especially designed to lead to nuclear modifications which would be indistinguishable from nuclear inclusions in virus diseases.

In 1926 Luger and Lauda [7] described and illustrated nuclear inclusions in salvarsan dermatitis which resembled very closely the type of inclusions in herpes. Cole and Kuttner,[8] however, took the view that before these could be accepted as an expression of salvarsan dermatitis, it would be necessary to demonstrate the absence of a filterable virus. In other words, they believed that when typical intranuclear inclusions are found, the presence of a virus is to be assumed unless its absence is proved experimentally. Cowdry,[9] on the other hand, has stated that the presence of intranuclear inclusions should not be taken

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† A slight residue was observed from salivary gland inclusions by Scott, Gordon H. Sur la localization des constituants minéraux dans les noyaux cellulaires des acini et des conduits excréteurs des glandes salivaires. *Compt. rend. Acad. d. sc.*, 1930, **190**, 1073.

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at face value as indicating the action of some filterable virus. He [10] also entertained the possibility that the inclusions may eventually be produced artificially without viruses. As nuclear inclusions form, the acidophilic material giving rise to them accumulates in the center of the nucleus and the basophilic chromatin migrates to the periphery and marginates on the nuclear membrane. Cowdry [10] has suggested that this process resembles to some extent chromatolysis in nerve cells and "chromatolysis by margination" in ovarian granulosa cells. Thinking that an enzyme might be involved he attempted without success to produce nuclear inclusions by the injection of nuclease prepared by Dr. D. J. Kooyman (personal communication).

Cowdry and Kitchen,[11] in testing the specificity of the nuclear inclusions in vesicular stomatitis, injected trypsin, pepsin, hydrochloric acid and various other fluids intradermally into the footpads of guinea pigs, but no artificial nuclear inclusions closely resembling the type inclusions of herpes were produced.

Of considerable significance are certain experiments on nerve cells. In 1929 Akiyama,[12] in a paper of which I have seen only reviews, reported a shrinkage of nucleoplasm away from the nuclear membrane, so that the central mass of material became surrounded by a clear halo something like that which often envelops a typical nuclear inclusion. He observed this modification as a result of subjecting nerve cells to galvanic stimulation plus ammonium ion. The following year Heinbecker and O'Leary [5] described, independently of Akiyama, at a meeting of the American Association of Anatomists, comparable nuclear alterations in several types of nerve cells after electrical excitation of their processes. They remarked upon the resemblance between the centrally placed chromatin masses and nuclear inclusions in virus diseases. In 1931 Davenport, Ranson and Terwilliger [13] discussed the observations of Akiyama, and of Heinbecker and O'Leary, and presented the results of their own experiments on the influence of drying and of hypertonic salt solutions on nerve cells. By these means a similar central clumping of nuclear material was produced which they illustrated. They suggested that ". . . the nuclear inclusions observed pathologically may be the result of disturbed osmotic conditions in the cell." Lee,[6] then, injected cats intravenously with a number of solutions calculated to alter osmotic relations and discovered various nuclear modifications but did not claim " . . . that any of the changes are identical with those caused by viruses though they resemble them in certain particulars."

More recently Cowdry and Scott,[14] while investigating renal lesions in monkeys given activated ergosterol 10,000 x, observed that nuclear inclusions were more frequently met with in the tubular cells of treated than of control animals. Since they occurred in 12 out of 16 treated, as contrasted with 1 out of 10 normal controls and 18 out of 107 pathological controls consisting of monkeys employed in the laboratory in a variety of experiments but not given activated ergosterol, the authors concluded that treatment "may have activated or intensified a process already latent in the kidneys. . . ."

In addition to chance observation of inclusions in the course of experiments and to purposeful attempts to produce them in the absence of virus, it is necessary to mention reports of their presence in tissues not subjected to any unusual procedure whatsoever. It is conceivable that the nuclei in normally functioning cells, which must adjust themselves to their particular environments (osmotic and otherwise), may on regular occasions be modified in such a way that they look as if they had been subjected to virus action. Confirmed observation of

this phenomenon would have a bearing on the principal problem of the significance of nuclear inclusions in virus diseases almost as direct as their experimental formation. Acidophilic intranuclear bodies, reminiscent of those in Borna disease and poliomyelitis, but not much like those in herpes — to which it is always necessary to refer as if to a standard — have indeed been repeatedly recorded in the epididymis [15-18] and in the nucleus supra-opticus and paraventricularis of the midbrain.[19, 20]

Obviously, however, the value of each and all of the above mentioned findings depends upon the precise degree of similarity between the structures noted and nuclear inclusions proved to be brought about by viruses.

My thanks are due Doctors E. V. Cowdry and James L. O'Leary for encouragement and advice.

Normal Structure of the Nuclei Selected for Experiment

Although nerve cells of various sorts were examined, Purkinje cells of the cerebellum of cats were chosen as the principal material because they are conveniently disposed in a single layer, are of fairly uniform size and shape, and possess large nuclei.

After ordinary fixatives, such as the fluids of Zenker, Carnoy, Bouin and Regaud, and coloration with hematoxylin and eosin or by Giemsa's stain, by far the most conspicuous nuclear component is the nucleolus (Figs. 1–3). Usually there is only one per nucleus and it is called an amphinucleolus for the reason that it is colored by both acid and basic dyes. The central core, which is more or less spherical, is acidophilic and on this is plastered in an irregular way a small amount of amphophilic material. In the ground substance between this amphinucleolus and the nuclear membrane a few tiny particles of acidophilic and basophilic material are generally visible. As a rule, the nuclear membrane colors only with "acid" dyes. The Purkinje cell nuclei resemble those of other large nerve cells in the spinal ganglia, anterior horns and other locations in being much less basophilic than most nuclei of corresponding size of non-nervous tissues.

Very little is known of the actual structure of the Purkinje cell nuclei *in vivo*. Examination of living cells by the method of dark-field illumination reveals no particles of nuclear material between the nucleolus and the nuclear membrane. The nucleolus, as in fixed material, is the most conspicuous nuclear component and the only one the existence of which has been demonstrated in living inter-

kinetic nuclei by the method of microdissection.[21] Bensley,[22] however, upon examining the nuclei of several different kinds of cells, after preparation by Gersh's [23] modification of the Altmann freezing and drying technique, concludes that "nuclei which are optically homogeneous in the living state are not in fact homogeneous but exhibit a chemical structure due primarily to the segregation of the chromatin in special locations in the interior of the nucleus." Preparations of the cerebellum made by this new technique showed particulate material in the nuclear ground substance, as Bensley has reported, and of which no indication is visible when viewed by direct or oblique illumination. At present each investigator must decide for himself which method is likely to reveal the true condition of affairs in the nuclei of living nerve cells — that of Altmann or of direct study of unfixed, still living tissue.

Care was taken to establish the range of variation in nuclear structure in a single individual and in different individuals, which could be regarded as the principal control. Tissues were excised from as nearly the same part of the vermis as possible and the Purkinje cells from the crest of each folium were chosen for study. Most of the variation in a single individual was in the arrangement of both acid and basic staining chromatin granules. In some nuclei these were distributed in an even fashion in the area between the nucleolus and the nuclear membrane; while in others most of them were concentrated in the zone around the nucleolus. Fine strands of acid staining material often stretched between these and a few particles just within the nuclear membrane. This left the intermediate zone relatively clear.

Sections of the cerebellum from 103 cats were examined. Twenty-five were normal. The others had been used in the laboratory for experimental purposes. Included in them were some that had undergone different degrees of vascular occlusion, for which I am grateful to Doctor Louis D. Tureen; others had received injections of various solutions directly into the pancreas or liver. In the Purkinje cells from this group of 103 animals there was a variation in both the amount and distribution of intranuclear chromatin, as well as the size and shape of the nuclei. Some nuclei were small and contained small clumps of both acid and basic staining substance, while others were larger and possessed scarcely any basophilic particles and a minimum of acid staining ones. In sections from one animal

the smaller nuclei would predominate while in another both large and small were present in about the same percentage. In all of the tissue examined approximately 65 per cent of the nuclei were of the small variety. In both types of nuclei, of these control sections, fine strands connected the acid and basic staining granules with the nuclear membrane, leaving no clear area between the two.

Intranuclear Aggregates Produced Experimentally

The experiments are listed in Table I. The injection of the various solutions was made with a syringe or burette connected directly with the femoral vein. The hypertonic solutions consisted of glucose, glucose plus acacia, sodium chloride and bicarbonate. Distilled water was injected as the hypotonic solution. All cats used in these experiments, including the controls, were killed by ether and exsanguination with the exception of those that died during the process of injection.

Small pieces of tissue were removed and fixed with 10 per cent formalin, Zenker's fluid containing 5 per cent acetic acid, 10 per cent formalin, and a solution containing 9 parts of absolute alcohol and 1 part formalin. They were then dehydrated, cleared, embedded in paraffin, sectioned at 5 microns and stained with hematoxylin and eosin or Giemsa.

(*A*) *Glucose*

Histological examination of tissues from Cats 1 and 2, which were killed immediately following the intravenous injection of glucose, showed definite nuclear alterations in cells of the spinal and sympathetic ganglia and spinal cord, Purkinje cells of the cerebellum, and in pyramidal cells throughout the thickness of the cerebral cortex. No comparable alterations were seen in sections of pancreas, spleen, liver, adrenal, lymph node, lung, thyroid, parotid and submaxillary gland from 6 animals that had received glucose intravenously. Tissues of the others, given glucose, were not examined.

In the Purkinje cells the nuclear material formed a fairly compact mass about the nucleolus as indicated in Figure 4. The mass was separated from the nuclear membrane by a large clear zone, strongly suggestive of the "halo" about the intranuclear inclusions in virus diseases. Intermediate stages with threads reaching from

TABLE I

Summary of Experiments

Cat. No.	Solution injected	Period of injection	Time of death after injection
1	120 cc. 50 per cent glucose	1 hour, 15 minutes	Immediately
2	130 cc. 50 per cent glucose	1 hour, 30 minutes	Immediately
3	120 cc. 50 per cent glucose	1 hour	1 hour
4	130 cc. 50 per cent glucose	1 hour	1 hour
5	130 cc. 50 per cent glucose	1 hour, 15 minutes	2 hours
6	120 cc. 50 per cent glucose	1 hour	2 hours
7	120 cc. 50 per cent glucose	1 hour	3 hours
8	120 cc. 50 per cent glucose	1 hour	3 hours
9	130 cc. 50 per cent glucose	1 hour, 20 minutes	4 hours
10	120 cc. 50 per cent glucose	1 hour	4 hours
11	120 cc. 50 per cent glucose	1 hour	24 hours
12	130 cc. 50 per cent glucose	1 hour, 30 minutes	3 days
13	120 cc. 50 per cent glucose	1 hour	5 days
14	120 cc. 50 per cent glucose plus 12 per cent acacia	2 hours	Immediately
15	120 cc. 50 per cent glucose plus 12 per cent acacia	2 hours, 30 minutes	Immediately
16, 17	150 cc. 30 per cent sodium chloride	1 hour, 30 minutes	Immediately
18, 19, 20, 21	50 cc. saturated sodium bicarbonate in distilled water	Animals died during injection	
22, 23, 24	300 to 400 cc. distilled water	2 hours	Injection continued until death

the shrunken chromatin mass to the nuclear membrane were seldom seen. The nucleoli were smaller than those of normal controls, stained heavily with hematoxylin and often could not be differentiated in staining qualities from the shrunken mass of nuclear material by which they were surrounded.

In Cats 3 and 4, which were killed 1 hour after the injection of glucose, nuclear modifications similar to those seen in Cats 1 and 2 were noted. As represented in Figure 5, the chromatin granules in the nuclei of the Purkinje cells formed a dense mass about the nucleolus. Both cells and nuclei were slightly larger than those of normal controls (Table II). The nucleoli were likewise swollen and vacuolated. Sometimes they could not be distinguished because they were completely masked by the nuclear material about them. The measurements were made in the following manner:

Fifty Purkinje cells from the cerebellum of a normal cat and of cats killed immediately and 1, 2, 3 and 4 hours after the injection of glucose were measured by the method of Scammon and Scott.[24] With the aid of a camera lucida the cells were outlined with India ink on "Kodaloid, No. 3" (Eastman). The outlines were cut out, placed in a desiccator for 24 hours and then weighed. The first row of figures in Table II gives the average weight of the cellular outlines in grams. These show that there is an initial decrease in cell volume immediately after injection, followed by an increase which reaches a maximum at 2 hours, and by 4 hours after injection is again within the range of normal variation in the material.

The greatest diameter of 100 Purkinje cell nuclei from the same 5 cats was measured in microns by an ocular micrometer. The second row of figures in Table II gives the averages. They show that there is an initial decrease in the size of the nuclei, followed by a gradual rise which reaches the maximum 2 hours following injection. By 3 hours after injection there is a marked decrease followed by a rise to normal within 4 hours.

The most pronounced swelling of both cells and nuclei was seen in tissues from Cats 5 and 6 which were killed 2 hours after the administration of glucose (Table II). In these cells (Fig. 6) the nucleoplasm formed an irregular granular mass about the nucleolus as in those animals killed earlier than 2 hours after injection. The intranuclear mass remained separated from the nuclear membrane by a "halo." The nucleoli, when they could be distinguished, were enlarged, vacuolated, and did not stain as heavily with hematoxylin and eosin as those of animals killed 1 hour after injection.

In sections from Cats 7 and 8, which were killed 3 hours after injection, the nuclei were much smaller than in the 2 hour animals and

slightly smaller than those of normal controls (Table II). It was apparent (Fig. 7) that the nuclear wall had partially collapsed, allowing the nuclear material to fill the entire nucleus, thus obliterating the "halo." The nuclear chromatin was grouped in large clumps throughout the nucleus and strands of nuclear material adhered to the nuclear membrane. The cells had likewise shrunken, leaving a large, clear, pericellular space. The nucleoli remained slightly enlarged and were plastered with chromatin particles.

Examination of the nuclei of Purkinje cells from Cats 9 and 10, which were killed 4 hours after injection (Figs. 8, 9 and 10), and a

TABLE II

Variation in Size of Cells and Nuclei

Condition of animal	Average weight of cellular outlines	Average nuclear diameter
	gm.	*μ*
Normal	0.6147	11.3560
Killed immediately after injection	0.5563	10.9384
Killed 1 hour after injection	0.7698	11.9455
Killed 2 hours after injection	0.9287	12.4375
Killed 3 hours after injection	0.7161	11.2260
Killed 4 hours after injection	0.6427	11.5380

comparison of these with normal controls showed practically the same distribution of chromatin and size of the nuclei as in normal controls.

In Cats 11, 12 and 13, which were killed at 1, 3 and 5 day intervals after glucose injection, the deposition of nuclear chromatin showed no more deviation from the mean than did the normal control material from different individuals.

Five micron sections of the material, which had been fixed in 90 per cent alcohol containing 10 per cent formalin, were incinerated by the method of Policard,[25] as modified by Dr. Gordon H. Scott,[26] to whom I am much indebted for aid in this connection. The normal ash content of the nerve cell nucleus was compared with that of animals killed immediately and 1, 2, 3 and 4 hours after the injection of glucose. The masses of nuclear substance showed a high ash content in contrast with known virus inclusions which were incinerated in

this laboratory by the same method (Cowdry,[2] and L. E. and E. J. Rector [3]).

Thymonucleic acid, as identified by the Feulgen reaction (Cowdry [1]), was confined to the intranuclear mass. It was most apparent about the nucleolus in animals killed immediately, and gradually dispersed in those killed 1, 2, 3 and 4 hours after injection. Bensley [22] has shown that thymonucleic acid is evenly distributed in normal cells prepared by the Altmann-Gersh procedure.

(*B*) *Glucose Plus Acacia*

It is known that acacia, when injected with a glucose solution, tends to hold for some time the fluid that is drawn into the blood stream. To determine whether or not the mixture would produce the same effect on nuclei as a glucose solution, 2 cats were injected with 120 cc. of 50 per cent glucose and 12 per cent acacia. In sections from these cats (14 and 15) nuclear alterations, like those of glucose-injected animals, occurred in the cells of spinal ganglia, anterior horns of the spinal cord, Purkinje cells of the cerebellum and in pyramidal cells throughout the thickness of the cerebral cortex. No sympathetic ganglia were examined. In most of the altered nuclei there was less shrinkage of the nuclear material and more strands of chromatin connecting the shrunken nuclear mass with the nuclear membrane than in glucose-injected animals.

(*C*) *Sodium Chloride*

Tissues from Cats 16 and 17, which were killed immediately following the injection of sodium chloride, exhibited nuclear changes similar to glucose and glucose plus acacia-injected animals, except that the nuclear chromatin was more acidophilic and less concentrated about the nucleolus than in the animals killed immediately after glucose injection.

(*D*) *Sodium Bicarbonate*

Fifty cc. of a saturated solution of sodium bicarbonate in distilled water were injected into each of Cats 18, 19, 20 and 21. All died during the process of injection. Microscopic examination revealed nuclear alterations in the nervous system comparable to those seen in the glucose-injected animals. No changes were noted in other or-

gans examined, such as liver, pancreas, spleen, adrenal, kidney and submaxillary gland. The cells of Purkinje were the only cells of the cerebellum affected. Their shrunken nuclear chromatin was finely granular and distinctly more acidophilic than that of the glucose treated animals. In the slightly swollen nuclei of cells from the anterior horns, spinal and sympathetic ganglia, the nuclear substance also formed a shrunken granular mass about the nucleolus in which were deposited from one to four acidophilic staining droplets, slightly smaller than the nucleoli, with irregular edges. The nucleoli remained apparently unmodified.

(*E*) *Distilled Water*

Examination of tissues from Cats 22, 23 and 24, which were injected with between 300 and 400 cc. of distilled water until death, brought to light nuclear changes in the cells of the spinal ganglia and anterior horns of the spinal cord. No alterations were apparent in any of the cells of the cerebellum, cerebral cortex, liver, pancreas, spleen, testis, ovary, kidney, adrenal or submaxillary glands. The nuclei of the injured cells varied considerably. Some were swollen while others were shrunken with centrally clumped chromatin. Approximately 5 per cent contained one to three smooth, round, acidophilic staining bodies whose diameter never exceeded half that of the nucleolus. The acidophilic bodies closely resembled the nuclear inclusions described by Wolf and Orton [27] as occurring in human nerve cells in a variety of pathological conditions. Indeed it is doubtful whether they could be distinguished from those represented in Wolf and Orton's Figures 2 and 3. They were seen only in fixed and stained preparations. Failure to observe them in fresh, still living nerve cells by transmitted light, or in the dark-field, may have been due to their rarity or absence in the particular cells examined. Their even outlines and obvious density were incompatible with the idea of formation by the coagulant or precipitant action of the fixative.

Comparison of Experimentally Produced Inclusions with Others in Virus Diseases

Since much work has been done in this laboratory on nuclear inclusions in nerve cells injured by the viruses of poliomyelitis and herpes, plenty of material was available for comparison. In addition, specimens of pseudorabies, Borna disease, B virus infection,

and rabbit encephalitis sent to Dr. Cowdry by Drs. E. V. Hurst,[28] I. A. Galloway,[29] A. B. Sabin [30] and C. Levaditi, respectively, were examined. A comparison of less value, because it related to nuclear inclusions in other than nerve cells, was made with preparations of the salivary glands of guinea pigs, moles,[31] hamsters [32] and rats,[33] of virus III disease, fox encephalitis,[34] a virus disease of parrots,[35] and many others generously contributed to the departmental collection.

It is easy to point out differences between inclusions caused by the injection of glucose and the true inclusions in virus diseases. Many will already have occurred to the reader. The experimental inclusions form very quickly — within 1½ hours after the beginning of injection — whereas in virus diseases they develop more slowly. For example, in pseudorabies they begin to appear 16 hours after virus inoculation (Hurst [28]), and in herpes they are reported after 24 hours (Goodpasture and Teague [36]).

There is a striking uniformity in the experimental inclusions not exhibited by those of the virus diseases. In any particular specimen they tend to be similar in all cells of the same type in contrast to the diversity in phases of nuclear inclusion formation often encountered in neighboring cells afflicted by viruses. In virus diseases it is not a case, simply, of nuclear response to some agent spread impartially by the blood stream to cells of the same kind, for there is local increase of virus in or near particular cells or groups of cells.

The nuclear modifications produced experimentally resembled the *early* changes in certain virus diseases (pseudorabies and rabbit encephalitis) in that the nuclei containing the inclusion bodies showed considerable swelling and a wrinkling of the nuclear membrane. When most conspicuous — from 1 to 2 hours after injection — the experimental inclusions are comparable with those of virus etiology in being separated from the nuclear membrane by a clear halo of material which does not color with acid or basic dyes; but the composition of the experimental inclusions themselves is not exactly the same as that of virus inclusions. Thus, in pseudorabies, herpes, B virus infections and rabbit encephalitis, the nucleoli are but slightly modified, retain their central position and are more or less obscured by the accumulation of material about them.

The material, which clumps about the centrally placed nucleolus to form the experimental inclusions, is irregularly particulate. Never has it been seen to be made up of particles as uniformly granular as

those in virus diseases (yellow fever, for instance). Chemically, also, there seems to be a difference. In the experimental inclusions the material comprises some basophilic (or amphophilic) as well as acidophilic particles. In virus diseases, on the other hand, all or nearly all of the basophilic particles shift to the nuclear membrane against which they marginate, leaving the inclusion wholly or almost entirely acidophilic. The intranuclear inclusions in the salivary glands of moles do stain with basic as well as acid dyes but they are exceptional.

These inclusions, caused by glucose injections, are very temporary modifications. The nuclei soon regain their normal structure. But in virus diseases nuclei possessed of inclusions are apparently on the way to inevitable death and degeneration. The experimental inclusions are not visible in fresh, unfixed preparations, whereas those in yellow fever and herpes are clearly visible with the aid of good optical equipment. This means that in the still living Purkinje cell nuclei some modification has taken place that leads to a different response to fixatives than would occur otherwise. Perhaps the density, or the coagulability of the perinucleolar nucleoplasm has been increased, but this is pure speculation.

The acidophilic masses, found in the nuclei of anterior horn and spinal ganglion cells of distilled water-injected cats, are less definite modifications and perhaps of little significance. They fall in Cowdry's[37] class of type B inclusions (which he says are not so definitely associated with virus action as type A inclusions, of which herpes is the leading example), because they usually occur in a nucleoplasm which shows no other noticeable change and there is certainly no evidence of margination of basophilic chromatin. In their size, arrangement, staining reaction and number they approximate closely the nuclear inclusions in poliomyelitis, as illustrated in Hurst's[38] Figures 4–15. By the same features they resemble the nuclear inclusions in Borna disease (equine encephalomyelitis[39]), but correspond rather more closely to the figures presented by Nicolau, Dimangesco-Nicolau and Galloway[29] than those of Hurst, for the nuclear inclusions illustrated in the former have the appearance of being rather more granular and less hyaline than in the latter. The experimentally induced inclusions were not supplied with halos. It is to be noted that the nuclear inclusions in these two diseases have not been reported in living cells, possibly because they are, like the

artificial ones, not very numerous. Though enumerating these similarities, it is unsafe to suggest identity in respect to the mechanism, osmotic or otherwise, of production. There may be in fact a real difference, since the poliomyelitis and Borna disease inclusions require days to develop, while the experimental ones form in 2 hours or less, the animals always dying during injection of the distilled water. The last named are suggestive of small acidophilic nucleoli, or plasmosomes. However, one would not look for nucleoli to appear so quickly. No explanation is offered as to why they were not produced in the Purkinje cells, like the centrally placed clumps resulting from the injections of hypertonic solutions.

Discussion

The most obvious initial alteration in the glucose-injected animals is a slight decrease in nuclear volume. This is followed by an increase in volume and a final return to about the original size. The time relations of these changes are presented in Table II. White and Erlanger [40] demonstrated that the immediate effect of injecting a solution consisting of 18 per cent glucose and 25 per cent acacia was a marked increase in blood volume, which slowly returned to normal. Their blood sugar figures show that the excess of glucose is completely removed from the blood stream, and presumably from the tissue fluids, within a period of 2 hours. This time is not very different from the lapse of 4 hours required for the nuclear substance to return to its previous physical state after the injection of 50 per cent glucose. A delay is to be expected in the adjustment of altered nucleoplasm to the reestablishment of normal conditions in the blood because the nucleoplasm is shielded by nuclear membrane, cytoplasm, cell membrane and tissue fluid.

Dehydration of the cell by exosmosis seems to alter the nuclear material in such a way that it is more readily acted on by coagulants. The addition of a minute amount of 0.1 per cent acetic acid causes the fresh nuclei of glucose-injected animals to assume the typical picture observed in fixed material. Lewis,[41] in her work on cultures of connective tissue from chick embryos, observed that by adding small amounts of each of several acids (including hydrochloric and acetic of pH 4.3) tiny granules were produced in nuclei previously appearing homogeneous. When this "gelation" was allowed

to continue the granules became progressively larger and finally a coarse reticulum with occasional larger masses resulted. She makes no mention of a change in the size of the nucleus. When the acid was washed off the nucleus returned to normal but, if the process was repeated several times, the cells soon died. Evidently the concentration of acid in the fixing fluid employed in these experiments was much stronger than that used by Lewis in the reversible gelation of tissue cultures. Substitution, in my experiments, of a fixative devoid of acid resulted in less pronounced granular aggregates in the nuclei. If Lewis had fixed, sectioned and stained cells whose nuclei had undergone gelation there is a chance that nuclear modifications, more like those caused by glucose injection, plus the technique, would have been formed.

Of particular interest is the distribution of the nuclear changes that took place in the cerebellum following glucose injection. They were limited to the nuclei of the Purkinje cells. The smaller and more chromatin-rich nuclei of the granule cells were not altered; nor were the nuclei in the other organs examined (liver, submaxillary gland, testis, ovary, adrenals, and so on), although viruses are known to produce typical inclusions in them. It seems possible that this difference in reaction may be due to the difference in amount of basophilic chromatin, relative to nuclear volume, possessed by chromatin-poor and chromatin-rich types of cells. An interesting parallel, with the viruses, is the fact that no typical virus inclusions have been described in lymphocytes, which possess an abundance of basophilic material, though they are formed in other types of nuclei which contain less.

Weed and McKibben [42] found internal changes, recognizable histologically, in the brains of animals that had been given intravenous injections of hypertonic solutions and that had not been trephined. There was a marked clear space about the nuclei of many cells. The nuclei appeared condensed, the chromatin aggregated. But in animals whose skulls had been opened so that the brains could undergo changes in volume, these histological changes were not demonstrated. For this reason the authors conclude that the alterations are due to increased intracranial pressure, stating however: "That there may be in these brains fundamental histological and cytological differences not revealed by the methods employed, is probable, but further work is necessary to establish such differ-

ences." That the intracranial pressure was not the factor causing nuclear modification following intravenous administration of glucose is evident, because similar changes were produced in the nuclei of sympathetic ganglion cells (Fig. 11) which are not boxed up in an unyielding bony case. They are to be seen also in spinal ganglion cells (Fig. 12).

The possibility that the earlier nuclear changes in pseudorabies, herpes, B virus and rabbit encephalitis are associated with the loss of water from the nucleus is appreciably strengthened by their similarity to those produced by the intravenous injection of hypertonic solutions. The early nuclear changes in Rift Valley fever and yellow fever are likewise accompanied by a loss of water. Findlay [43] noted that, in Rift Valley fever, with the growth of inclusions the nuclear membrane becomes crumpled and shrunken as if fluid had been lost by osmosis. The illustrations of Cowdry and Kitchen [11] apparently show the same phenomenon in experimental yellow fever in monkeys.

The change in the nucleoplasm of large nerve cells, evoked by the intravenous injection of hypertonic solutions and which is indicated after fixation and staining by the appearance of these interesting intranuclear masses, takes place promptly and lasts for only a short time after the stimulus is removed. It very definitely falls short of the production of well formed inclusions characteristic of virus diseases and accompanied by disappearance of nucleoli. But the action of most viruses in the production of inclusions is not systemic; rather is it local and persistent, at least for a period of days. These experiments give no answer to the question whether a more enduring osmotic stimulus, focussed on limited cell groups and supplemented perhaps by cellular injury, which is sometimes thought [44] most helpful in enabling a virus to take hold, would actually lead to the formation of more satisfying intranuclear inclusions.

Summary

Two types of nuclear changes were produced: (1) by the intravenous injection of a hypertonic solution of either glucose, glucose plus acacia, sodium chloride or bicarbonate, and (2) by injection of a large quantity of distilled water. Changes of the first type were noted in the Purkinje cells of the cerebellum, anterior horns of the

spinal cord, spinal and sympathetic ganglia and in the pyramidal cells throughout the thickness of the cerebral cortex; while those of the second type appeared in cells of the anterior horns of the spinal cord and spinal ganglia. No nuclear alterations were noted in any other tissues examined.

Nuclear reactions produced by the hypertonic solutions resembled the early alterations in nerve cells following pseudorabies, herpes and B virus infection, while those produced by distilled water more closely resembled inclusions of the poliomyelitis and Borna disease type; but it was not possible to produce any nuclear inclusions which were so much like inclusions in virus diseases that they could be mistaken for them.

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DESCRIPTION OF PLATES

PLATE 25

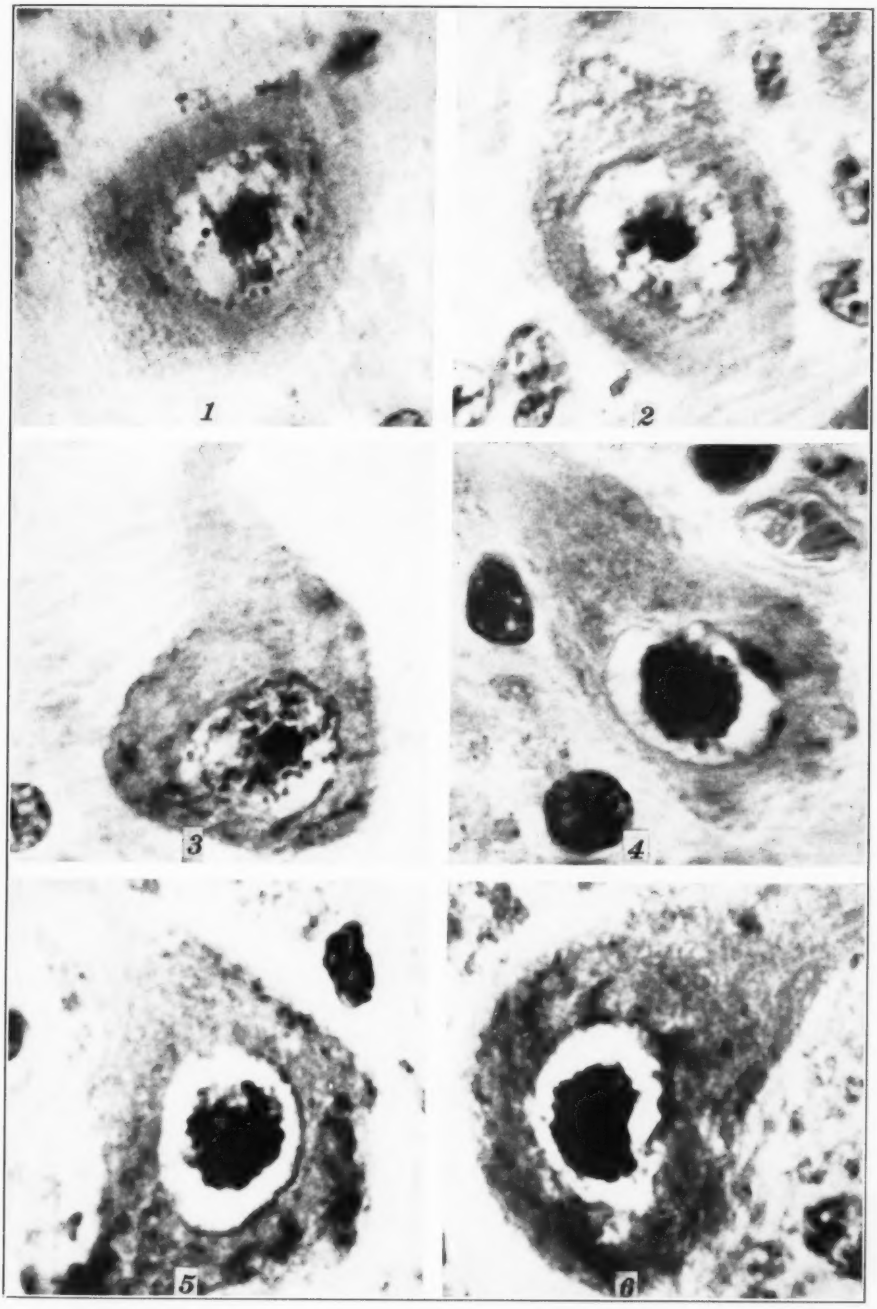
Photomicrographs at a magnification of 2200 diameters of Zenker-fixed and hematoxylin and eosin-stained sections from the cat's cerebellum, which illustrate the distribution of nuclear chromatin in Purkinje cells of apparently normal animals and in those killed at various intervals after injection of glucose.

FIGS. 1, 2 and 3. Illustrating the characteristic distribution of chromatin particles in control sections.

FIG. 4. Compact mass of nuclear substance enclosing the nucleolus from an animal killed immediately after glucose injection.

FIG. 5. In cats killed 1 hour after injection the nucleus is slightly swollen. The chromatin mass forms a less compact mass about the nucleolus than in Figure 4.

FIG. 6. Showing a greatly enlarged nucleus enclosing a shrunken chromatin mass from an animal killed 2 hours after injection.



Lee

Alterations Following Injections of Glucose

PLATE 26

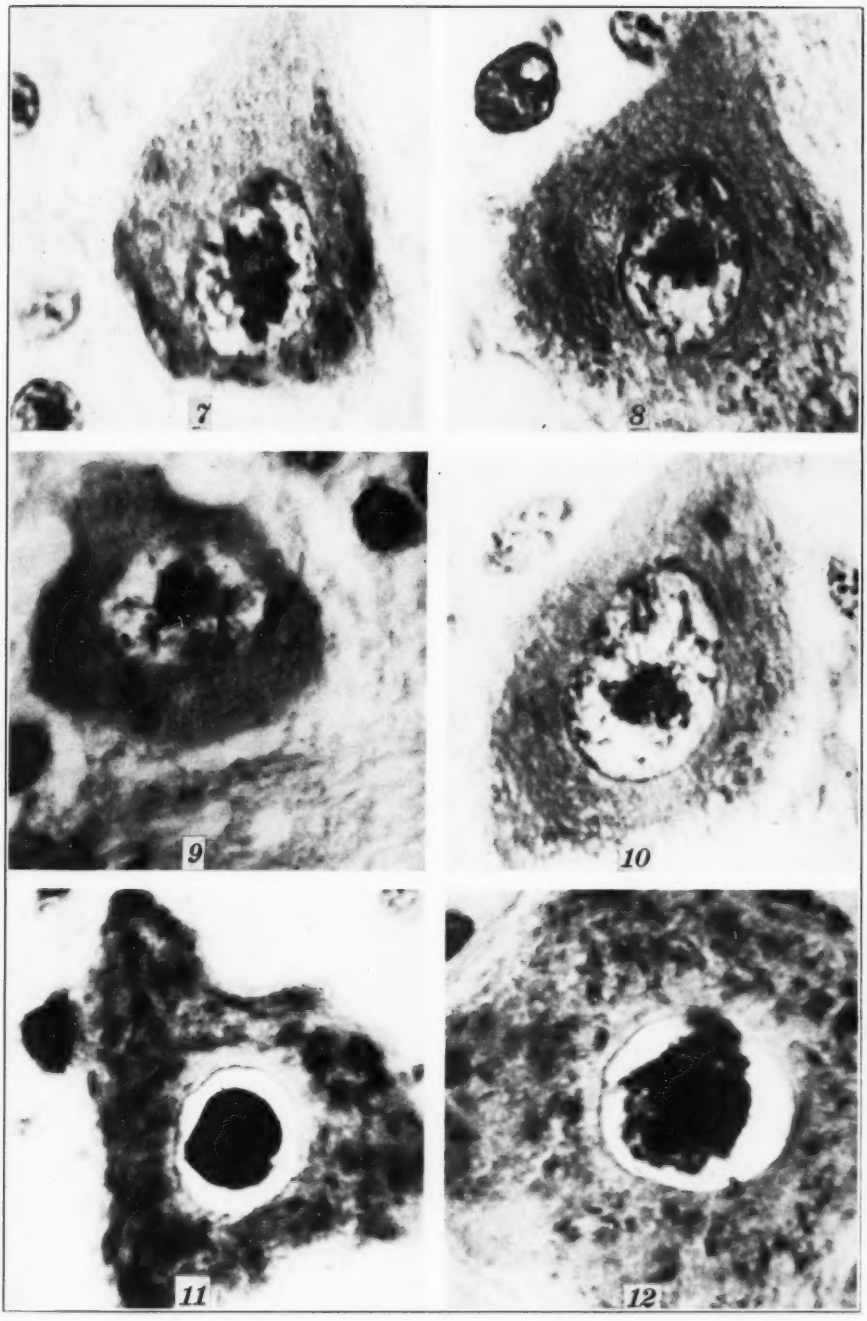
Photomicrographs at a magnification of 2200 diameters of Zenker-fixed and hematoxylin and eosin-stained sections of cerebellum, spinal and sympathetic ganglia from glucose-injected cats.

FIG. 7. Purkinje cell of the cerebellum from an animal killed 3 hours after injection. The nuclear membrane has partially collapsed, obliterating the "halo" seen in Figures 4, 5 and 6.

FIGS. 8, 9 and 10. Purkinje cells from the cerebellums of cats killed 4 hours after injection. A comparison of these with Figures 1, 2 and 3 shows that the distribution of nuclear chromatin is practically the same as that of the control sections.

FIG. 11. Sympathetic ganglion cell which shows shrinkage of nuclear substance when animal is killed immediately after injection.

FIG. 12. Shows shrunken nuclear mass in spinal ganglion cell of a cat killed immediately after injection.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DERMATOMYOSITIS *

A REPORT OF TWO CASES WITH COMPLETE AUTOPSY

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Dermatomyositis was originally described by Wagner in 1887 and almost simultaneously by Unverricht and Hepp. Steiner, in his thorough study, defined the disease as "an acute, subacute, or chronic disease of unknown origin characterized by a gradual onset with vague and indefinite prodromata followed by edema, dermatitis and a multiple muscle inflammation." Although the condition is a rare one, there have been about 85 cases described to date; many of these have not been confirmed by pathological study. The frequency of this condition is probably greater than is apparent, since it is a disease that may readily be overlooked, not only by the clinician but by the pathologist who does not examine voluntary muscle as frequently as he might in general autopsies.

CASE REPORTS

CASE 1. G. B. (N. I. 12986), a white male, 53 years of age, was admitted to the Neurological Institute in June, 1932. Three months before admission he developed a dermatitis which began with redness and itching about the finger nails. It spread to the arms and was associated with a burning sensation and scaling. Within a week the forehead, eyelids, nose and ears had become similarly affected. A sensation of warmth and itching was particularly noticeable in the skin lesions at night. Two and a half months before entry to the hospital he developed weakness of the arms and legs which was preceded by slight pains in the extremities. The neck muscles became involved so that he had difficulty in holding up his head. This weakness grew progressively worse and he had to stop working 1½ months before admission. He next began to suffer from difficulty in speech and swallowing, frequently regurgitating food through the nose. Night sweats appeared and the skin became dry and painful over the buttocks and back. He was confined to bed during the month before admission, any exertion causing him to become dyspneic. The dysphagia increased and he had difficulty in coughing up the large amounts of mucus that collected in his throat. There was a sense of tightness in the skin of the arms and legs. There had been a loss of 5 to 6 pounds during the 3 months of illness.

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On admission the patient appeared chronically ill. The temperature was 99.4° F., the pulse rate 96 per minute, respiratory rate 24 per minute and blood pressure 136/68. There was a red, dry, scaling pruritic rash present on the forehead, eyelids, ears, chest, neck and extensor surfaces of the arms and legs. There was extreme muscular weakness with considerable atrophy involving all the muscle groups. Dyspnea and dysphagia were present, and he could not cough effectively. The blood count showed a hemoglobin of 82 per cent, 4,300,000 red blood cells and 24,000 white blood cells per cmm., with 85 per cent polymorphonuclears, 10 per cent lymphocytes, 4 per cent large monocytes and 1 per cent eosinophiles. The urine contained a few white blood cells, but was otherwise negative. The spinal fluid findings were negative. There was no edema and the spleen was not enlarged.

Four hours after admission the patient's temperature rose to 104° F., although no new symptoms appeared and no new focal signs were found. The next morning he was somewhat better. His temperature was 102° F., and he was coughing less and swallowing more easily. Just after finishing luncheon, without any apparent difficulty, he suddenly became cyanotic, coughed a good deal, lapsed into stupor and died a few minutes later. No definite diagnosis was made.

Postmortem Examination

Gross Findings

At autopsy an extensive rash was noted over both forearms, arms and shoulders. The skin in these areas was scaling, roughened and slightly injected. It felt firm and was fixed to the underlying tissues. The eyelids were extensively involved in the same process, being covered by thick, dry white scales. There was no pitting edema. The thorax, abdomen and lower extremities were entirely free of skin lesions. All the skeletal muscles were very pale, being light grayish in color, and seemed normal in consistence. There did not appear to be any gross increase in fibrous tissue in them and no replacement by fat. The other positive findings were as follows:

A few localized fibrous adhesions were present in the region of the cecum and when these were separated about 5 cc. of thick, greenish yellow pus were disclosed in the retrocecal position lying directly posterior to the shortened, thickened and slightly injected appendix. The process was well localized.

The left pleural cavity contained about 150 cc. of cloudy yellow fluid. There was a delicate fibrinous exudate over the pleural surface of the lower lobe of the left lung. The bronchi of this lobe contained purulent material. There were many irregular areas of consolidation in the parenchyma.

The gall-bladder contained about fifty hard, jagged gall-stones.

Its mucosal surface was smooth and deeply bile-stained. At the extreme apex a rather hard nodule, covered by mucosa and measuring 1.5 by 1 by 1 cm., protruded into the lumen. On section it was finely granular and yellowish gray. It extended through the muscular coat but had not penetrated the serous covering. A similar nodule, measuring 2 cm. in diameter, apparently in a lymph node, lay adjacent to the cystic duct, which was patent.

The brain, spinal cord and meninges showed no gross abnormalities, externally or on section.

Histological Findings

Skin: On the surface of the skin there was a thick layer of cornified material containing considerable nuclear and some eosinophilic granular débris. The epidermis was considerably compressed and flattened over large areas and here the normal papillae were not in evidence. The basal cells of the epidermis showed marked vacuolization of their cytoplasm. No pigment was noted in the epidermis and only occasional, small pigmented cells were found in the corium. The dermis was thickened and its collagen masses appeared considerably swollen and somewhat hyaline. Just beneath the epidermis there were scattered lymphocytes, polymorphonuclear and large mononuclear leukocytes. There was a mild perivascular exudate of the same type, chiefly about the vessels in the outer portion of the corium. A similar infiltration was seen about the ducts of the sweat glands.

Muscle: Scattered perivascular infiltrations by lymphocytes, large mononuclears, plasma cells, a few polymorphonuclear leukocytes and occasional mast cells were present. There was diffuse infiltration as well. These infiltrative cells were found chiefly in the perimysium but were present, scattered, in the endomysium as well. Quite a number of muscle fibers showed loss of their transverse striations. Some fibers stained faintly and presented vacuolated zones which contained varying amounts of finely granular, eosinophilic material. Frequently there was a considerable multiplication of the muscle nuclei. The sarcolemma sheaths were occasionally raised over small areas, producing surface blebbing.

In the more severely affected muscles the perimysial infiltration was often quite intense. A great many muscle fibers were pale stain-

ing and showed loss of their striations. They were vacuolated and exhibited fragmentation of their fibrils. Frequently they were partially hyalinized. A considerable increase in the perimysial connective tissue was observed and some multiplication of sarcolemma cells. Fibrous replacement of degenerated muscle fibers had occurred and this fibrous tissue was at times diffusely infiltrated. Occasionally the sarcolemma sheaths appeared to be torn and muscle fibrils protruded through the tear. Irregular multinucleated giant cells lying in the course of degenerated muscle fibers were seen. These had a somewhat more bluish tinge to their cytoplasm than the neighboring muscle fibers and their nuclei contained more chromatin. They appeared to be an attempt at muscle regeneration. Considerable, finely globular neutral fat was seen in the degenerating muscle fibers. The small nerves in the muscle showed occasional perineurial infiltration similar to that in the perimysium, but no other changes. The muscle spindles appeared normal.

Other Tissues: Representative sections of the cortex, basal ganglia, brain stem and spinal cord revealed no abnormal histological findings.

The nodule in the fundus of the gall-bladder proved to be an early carcinoma of that organ. The mass in the lymph node near the cystic duct was the only metastasis.

The appendix exhibited the results of a suppurative inflammation and on its surface there was granulation tissue forming the wall of the periappendiceal abscess.

The lung showed a terminal lobular pneumonia.

CASE 2. M. M. (P. H. 377533), a white female, 47 years of age, was admitted to the Presbyterian Hospital on May 10, 1933. The initial symptom was a dermatitis which developed 8 months before admission, beginning on the palms. The skin at first was leathery in consistence; later, a generalized, red scaling eruption developed and the skin became thickened. This was diagnosed as dermatitis exfoliativa. She began to lose weight. Three months later the dermatitis began to clear up. Eight weeks before admission difficulty in articulation and swallowing developed. A right peritonsillar abscess was discovered at about this time and 4 cc. of foul pus evacuated from it. During the last 3 weeks before admission the patient grew progressively weaker. The impairment of speech and swallowing became more intense. She coughed frequently and ineffectively, much mucous secretion collecting in her throat. Weakness of the legs and back developed. She was able to take only fluids and had lost 68 pounds since the onset of illness.

On admission the patient appeared chronically ill and showed evidence of a loss of weight. Her speech was unintelligible, accomplished with considerable

exertion, and followed by marked fatigue and dyspnea. There was mucus in the throat and trachea. Only the cervical lymph nodes were palpable. The lungs, heart and abdomen were negative. There was no clubbing of the fingers, no cyanosis and no edema. The skin eruption had entirely cleared save for some residual thickening, especially on the feet, and a little scaling at the elbows. Deep reflexes were diminished. The diaphragm, intercostal and abdominal muscles were weak. The temperature was 99.2° F. on admission, rose to 100.6° on the 2nd day and then returned to normal, to remain there. Blood pressure was 110 systolic and 80 diastolic. The pulse rate varied from 94 to 102 and respiratory rate from 22 to 40 per minute. The hemoglobin was 94 per cent, red blood cells 4,900,000 per cmm. and white blood cells 13,700, with 80 per cent polymorphonuclear and 8 per cent mononuclear leukocytes, 1 per cent transitional forms and 1 per cent lymphocytes. The Wassermann test was negative in the blood and spinal fluid. The spinal fluid was not abnormal. A neurological examination revealed no sensory changes. There was weakness of the palate and an impaired swallowing mechanism. The vocal cords were not paralyzed. The intercostal, diaphragm and abdominal muscles and the extensor quadriceps muscle groups were weak.

The patient had increasing difficulty in breathing and swallowing. She was placed in a respirator but failed to improve. On the 4th day in the hospital she became irrational and maniacal and died after several hours of excitement. The clinical diagnosis was not definitely established, but was thought to be bulbar paralysis terminating a poliomyelitis.

Postmortem Examination

Gross Findings

At autopsy the body was found to be well developed and in fair nutrition. Moderate scaling and thickening of the skin of the neck, hands, forearms, elbows and soles of the feet (most marked on the latter) were present. There was no evidence of edema. The temporal, pectoralis minor and intercostal muscles were very pale and grayish pink. The psoas muscles, right rectus femoris and muscles of the back were also paler than normal, but the remainder of the skeletal muscles examined appeared grossly normal. The rest of the gross examination was essentially negative save for a moderate degree of congestion of the viscera. The central nervous system, brain and spinal cord, revealed no gross abnormalities.

Histological Findings

Skin: Hyperkeratosis was present. The outermost cell layers contained pigment. The vessels of the corium showed a mild perivascular lymphocytic infiltration.

Muscle: An intense inflammatory reaction was observed in the perimysium and to a lesser extent in the endomysium. The cells

TABLE I

Analyses of Muscle

(Case 2)

Muscle	Moisture	Creatine		Total nitrogen		Ratio creatine N_2
		Wet	Dry	Wet	Dry	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Right rectus femoris	79.64	0.098	0.482	...	...	...
Left pectoral	76.37	0.255	1.077	2.85	12.08	2.86
Right psoas	81.47	0.253	1.365	2.65	14.30	2.48
Right back muscle .	75.86	0.109	0.452	...	...	...
Left rectus	74.82	0.283	1.125	...	...	...
Left intercostal	71.64	0.136	0.480	...	...	...
Diaphragm	78.25	0.070	0.322	...	...	...

Case of Dermatomyositis Cited by Steinitz and Steinfeld

Diaphragm	0.282	
Adductor femoris	0.310	
Biceps	0.188	Different parts of same muscle
	0.143	

Normal Cases (Adults) Cited by Bodansky

Diaphragm	0.331	3 different cases
	0.309	
	0.309	
Myocardium	0.261	"
	0.257	
	0.220	
Psoas	0.485	"
	0.465	
	0.448	
Intercostal	0.220?	2 different cases (difficult to separate muscle fibers)
	0.266?	
Pectoralis major	0.447	3 different cases
	0.456	
	0.433	

consisted mainly of lymphocytes accompanied by a few plasma cells and eosinophiles and many mononuclear cells. The myofibrils could be seen in many of the muscle fibers, but the cross striations were often indistinct. Some fibers showed hyaline and others vacuolar

degeneration. Granular débris was present in many of the vacuoles. Frequently there was an increase in the muscle nuclei, possibly a condensation, as well as in the sarcolemma sheath nuclei. Many of the muscle fibers appeared to be fragmented and their ends were retracted and coiled. In a few areas there were groups of large, elongated mononuclear cells with fibrils which were probably myoblasts. Some of these cells showed mitoses.

The changes were severe in the intercostal and back muscles examined, as well as in the tongue. They were moderate in the rectus femoris, psoas, rectus abdominis and temporal muscles.

No microscopic abnormalities were noted in representative sections of the brain and spinal cord.

Analyses of various muscles obtained at autopsy were made by Dr. Goettsch and the accompanying table gives the results. For comparison the results of a similar chemical study by Steinitz and Steinfeld are added, as well as Bodansky's figures (see Steinitz and Steinfeld) obtained in normal human muscle. The creatine content is observed to be lowered in the diseased muscles. The degree of this diminution appears to correspond roughly to the extent of involvement of the muscles.

Discussion

Dermatomyositis occurs equally in both sexes and is more frequent in middle life. A little less than one-third of the reported cases, however, occurred in the first two decades. The disease is more common in the temperate zone and there is no definite seasonal incidence, although possibly its inception is more frequent in the winter months.

The clinical picture may be quite variable. There may be an initial period of malaise, generalized pains, insomnia and anorexia, or the disease may begin with acutely painful muscles, usually in the extremities, but in other cases the skin lesion may occur first. The muscles become swollen, painful and definitely weak. There is no rule as to the areas first involved, but often the upper extremities, shoulders and face are the primary site. The entire voluntary musculature as well as the muscles of deglutition and respiration may be involved eventually. A doughy edema of the skin is present which may produce a considerable increase in the size of the extremities and a mask-like facies. The skin lesion may consist of an erythema re-

sembling an erythema nodosum or multiforme, or the skin may appear tight and glistening. The skin lesions, however, may vary considerably in type. The mucous membranes may be involved in certain cases, producing a stomatitis with ulceration or an angina. There is usually a moderate fever, and not infrequently an enlarged spleen, with a tendency toward profuse perspiration. As the disease progresses, muscular weakness becomes more marked and movement, both active and passive, impaired. The muscles of respiration and mastication may become involved and dysphagia and respiratory embarrassment occur. This may lead to asphyxia or an aspiration pneumonia. The mortality is fairly high, approximately one-half the cases ending fatally. Of the 28 cases collected by Steiner, 17 were fatal. Those cases that tend to recover may run a chronic course and the marked muscular damage may lead to atrophy and fibrosis, contractures, and even to calcification. Residual changes may be noted in the skin which remains thickened and often pigmented. This cutaneous change frequently resembles that of scleroderma, and Klingman and others suggest that scleroderma may be preceded by this condition. Occasionally the disease has an intermittent character. It may be fatal in from 1 to 4 weeks or death may occur after nearly 2 years. Recovery or death usually takes place in a few months.

Clinically the 2 cases reported here showed some atypical features. In neither of the 2 cases was pain an outstanding symptom. The pain in this disease is of course due to the myositis. That there were widespread inflammatory lesions of the musculature in both our cases is certain from the pathological examination. It may be that the intensity of the pain in other instances of the disease is dependent on the degree of involvement of the intramuscular nerves. This was relatively slight in both of our cases.

In each instance the dermatitis apparently preceded the myositis and neither patient at the time when in the hospital exhibited any of the doughy edema described in other cases. It may be that this was present earlier in the disease, although no clear history of it was obtained from either patient. The edema was slight in Jacoby's case (see Steiner). The leathery, tight, somewhat shiny skin adherent to the underlying tissues so consistently reported as a late stage of the skin lesion in dermatomyositis was present in both of our cases.

In the first patient considerable muscular atrophy was present. This has been mentioned as a sequel of the disease, but not sufficiently indicated as a possible part of the syndrome. It is obvious from histological study of the musculature that there is widespread degeneration of muscle fibers with a consequent loss of muscle substance. When the process is a very rapid one, as it was in the first case, it is understandable that a considerable muscular atrophy may develop quickly.

Pathology

The disease process is an inflammation, commonly with degeneration, of the skin and striated musculature including the muscles of deglutition and respiration. Although the disease is most conspicuous in the skeletal musculature, in a small percentage of cases the heart muscle is also involved. Smooth muscle fails to develop the lesions.

Grossly the skin lesions may be quite variable in character. They may appear as an erythema, erythema nodosum, erythema multiforme, erysipeloid lesion, roseola or urticaria. They are most frequently accompanied by a non-pitting edema. Later the skin may scale and become brownish, leathery, tight, shiny, atrophic and attached to the underlying tissues. As mentioned above, the end result may be a scleroderma. Microscopically the skin shows flattening of the papillae and vacuolization of the cells in the basal layer. The dermis is edematous and the collagen fibers appear swollen and semihyaline. Mild round cell accumulations occur about the vessels and sweat glands. These cells are chiefly lymphocytes and monocytes and the infiltration is most common directly beneath the epidermis. Later there is desquamation of considerable cornified epidermis with thinning of the latter, the papillae may disappear over large areas and there is a widespread increase of collagen in the corium. The underlying fatty tissue secondarily affected by the inflammation may undergo some fibrosis.

Grossly the affected muscles are dull, grayish yellow, yellow or pale red in color, and firm and rubbery, or soft and doughy. They may have small scattered hemorrhages in them and be friable. Later they show much loss of substance and fibrosis, rarely with calcification. Microscopically the muscles show edema of their endomysium and perimysium with from mild to marked perivascular and diffuse

round cell infiltrations in the same membranes. The inflammatory cells include lymphocytes, large mononuclears, plasma cells, some polymorphonuclear leukocytes and occasional mast cells. In later stages the endomysium and perimysium are increasingly thickened. The muscle fibers show a loss of striation, fragmentation of fibrils, granular, hyaline or waxy degeneration, vacuolization or rupture. There may be marked irregularity in the size and an increase in the number of muscle and sarcolemma nuclei. Frequently there is blebbing of the sarcolemma sheath. Multinucleated cells may be present as evidence of attempts at regeneration.

The etiology of the disease is unknown. Because of the acute onset, the fever, angina, frequently enlarged spleen, sweats and muscle inflammation, it long has been suspected that it is an infection. A great variety of causative agents have been suggested. Unverricht suggested a gregarine; Langsteiner (see Steiner), a streptococcus; Bauer, a staphylococcus; Martinotti, a micrococcus; and others a variety of other organisms, but none of these has been found consistently in the disease. Strümpell called attention to the occurrence of tuberculosis in some of these cases and thought there was a possible relationship. Grunke reported an instance with a typical clinical syndrome in which the lesions were obviously tuberculous. The disease has been observed following upper respiratory infection, acute rheumatic fever, some of the acute infectious diseases of childhood and gastro-intestinal intoxications. More recently it was described in the presence of chronic suppurative foci following typhoid by Costanzi. It is possible that more than one agent may either produce this condition or be the predisposing factor. It is interesting that in our cases foci of infection were present; in the first case a periappendiceal abscess, in the second a peritonsillar abscess. Gram, methylene blue, carbol-fuchsin and Jahnel stains revealed no organisms. A careful search of our skin and muscle material for cell inclusions which would indicate the possibility of a virus origin of the disease yielded negative results. No fresh material for injection into animals was available.

Although it seems more probable that the condition is an infection, degenerative changes with secondary inflammatory phenomena in the skin and muscles due to toxins from some other primary condition are another possibility. Similar muscle lesions produced in guinea pigs and rabbits by Goettsch and Pappenheimer by the use

of special diets show many features of the muscle lesions of dermatomyositis. No definite evidence, however, of improper nutrition has been encountered in the known cases of this disease.

Steinitz and Steinfeld studied the creatine metabolism in a case of dermatomyositis and found it to be markedly affected. There was a constant creatinuria with a low total creatinine excretion, coupled with an inability to handle orally administered creatine. Some of the muscles showed a decreased creatine content. This was checked in our second case and the creatine content was below normal in each of the seven muscles examined.

In conditions in which muscle tissue undergoes degeneration it is known that creatinine excretion is lowered and creatine appears in the urine. The loss of creatine in the muscle is probably directly dependent upon the amount of muscle tissue destroyed.

Steinitz and Steinfeld refer to the case of "myositis fibrosa" of Bodansky, Schwab and Brindley which they think may have been a late stage of dermatomyositis. The creatine metabolism was studied in that case and the changes observed were similar to those found by Steinitz and Steinfeld in their case, except that there was no diminution in the total creatinine excretion. These chemical studies, although interesting, throw no light on the essential etiology of the disease.

Summary

1. Two cases of dermatomyositis are presented with autopsy reports. One was a male, 53 years of age, with a history of 3 months duration, and the other a female, 47 years of age, with a history of 8 months duration.

2. Each case showed widespread inflammatory and degenerative lesions of the skeletal musculature and less disseminated skin lesions. In 1 case there was an acute and in the other a chronic focal infection.

3. Atypical features, such as the slight pain, the small amount of edema and the advanced muscle atrophy in 1 case are pointed out.

4. The results of a quantitative determination of the creatinine content of the affected muscles in 1 case are given.

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DESCRIPTION OF PLATES

PLATE 27

FIG. 1. Round cell infiltration of perimysium and endomysium of striated muscle (Case 1). × 100.

FIG. 2. Degeneration and disappearance of muscle fibers. Edema and mild, diffuse, round cell infiltration (Case 1). × 100.

FIG. 3. Round cell infiltration in corium directly beneath epidermis. Flattening of papillae (Case 1). × 460.

FIG. 4. Vacuolization, especially of basal layer of epidermis. Edema of corium (Case 1). × 460.

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3 4

Wolf and Wilens Dermatomyositis

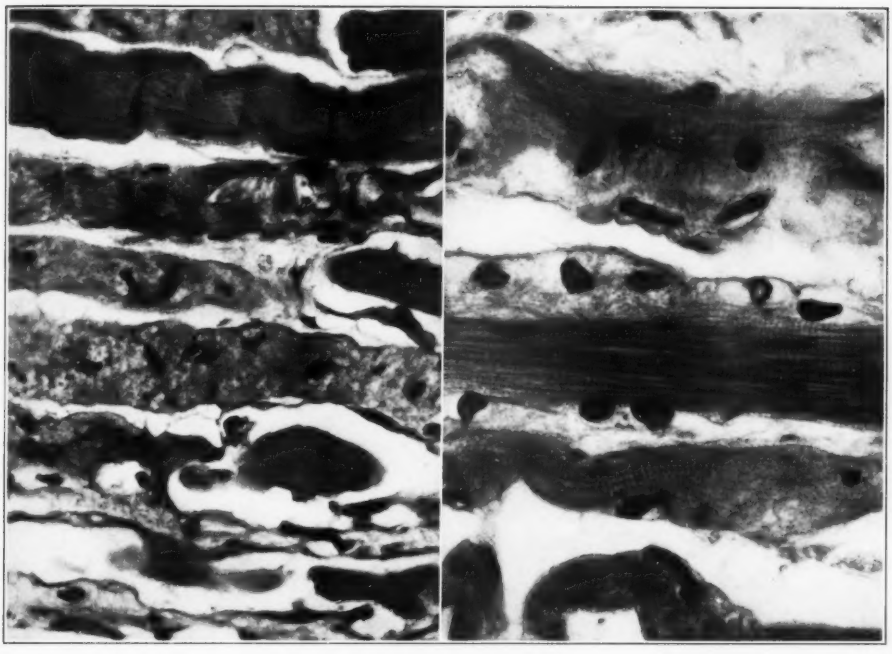
PLATE 28

FIG. 5. Normal muscle fiber at top. Other fibers all undergoing necrosis, illustrating granular, hyaline and vacuolar degeneration. Increase of muscle and sarcolemma nuclei (Case 1). × 460.

FIG. 6. Blebbing of sarcolemma sheaths of muscle fibers (Case 1). × 700.

FIG. 7. Multinucleated cells with deeply staining cytoplasm in degenerated muscle. Probable attempt at regeneration (Case 1). × 700.

FIG. 8. Cross-section of muscle fiber undergoing degeneration. Thickened endomysium and perimysium. Mild round cell infiltration (Case 2). × 700.



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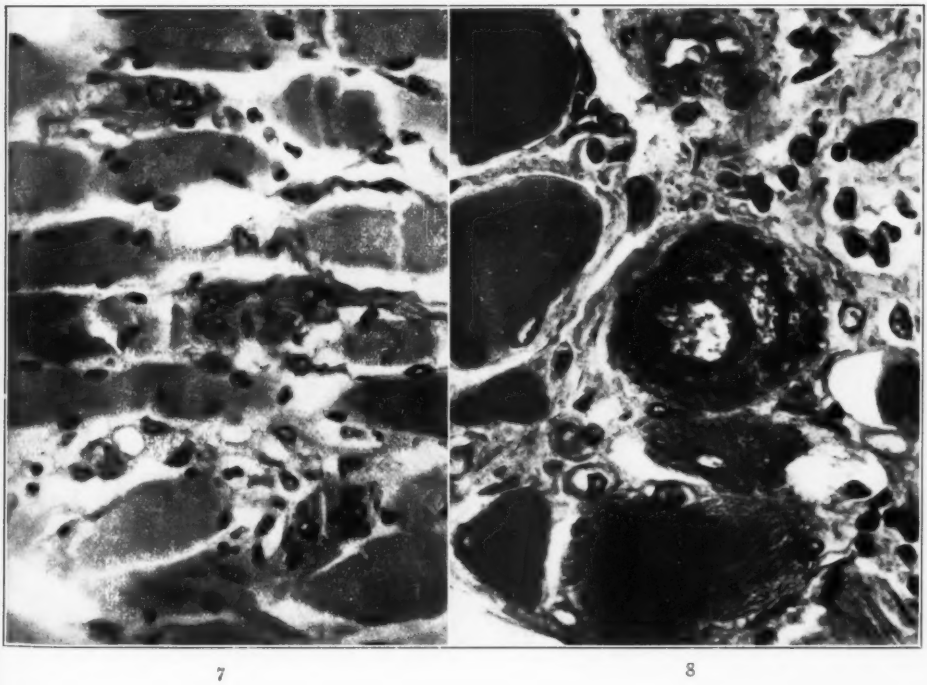

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Wolf and Wilens Dermatomyositis

SPONTANEOUS LEUKEMIA AND CHLOROLEUKEMIA IN THE RAT *

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Leukemia is known to occur in almost all of the common domestic and laboratory animals. It has repeatedly been described in fowl, cattle, horses, swine, canaries, dogs, cats, mice, guinea pigs and monkeys. The literature on this subject has been reviewed by Opie [1] and by Bayon and coworkers.[2] Apparently the disease has never been observed in rats. This hiatus is all the more surprising when the large number of rats under constant laboratory observation is considered.

Chloroma, or chloroleukemia, an uncommon condition in man, has been infrequently observed in animals. It has been reported in the pig by Claussen [3] and Joest.[4] Hill [5] mentions among the atypical cases in a large group of leukemic mice, 1 in which the lymph glands and tumor-like growths were bright green in color. This was classified as chloromyelosarcoma. Mathews [6] has described a form of fowl leukosis associated with local tumors composed of myeloid cells. He designated this condition as leukochloroma and related it to chloroma in man, although the tumor masses were described as chalky white. The green pigmentation, which has always been considered a prominent feature in this condition and from which the name is derived, was lacking.

The purpose of this report is to record 12 instances of leukemia developing spontaneously in an inbred strain of albino rats. Eleven of these were of the myeloid type and 1 of the lymphatic. Of the myeloid group, 4 showed a very striking light green pigmentation in all areas where leukemic cells were present in abundance and are, therefore, classified as chloroleukemia. The diagnosis of leukemia was based on autopsy findings, the criteria being leukemic changes in the bone marrow, spleen, lymph nodes and blood vessel contents, as well as infiltrations into the viscera.

* Received for publication October 3, 1935.

The rats, pure albinos of Osborne-Mendel stock, had been bred in the laboratory of Dr. H. C. Sherman of the Chemistry Department, Columbia University, for many generations over a period of years and were subjected to nutritional experiments.* In no case was the presence of leukemia suspected during life. Blood cell counts were not done.

The total number of deaths due to spontaneous causes in the colony over a period of about 20 months was 365. All the animals were subjected to a complete gross and microscopic examination. Twelve showed clear-cut evidence of leukemia, an incidence of 3.3 per cent. The most plausible explanation for the fairly high incidence of leukemia in this particular group is on an hereditary basis. Richter and MacDowell [7, 8] have emphasized the genetic factor in mouse leukemia. They showed that not only was there a marked difference in the incidence of spontaneous leukemia in several strains of mice, but also that mice of a strain with a high incidence were all readily susceptible to experimentally transmitted leukemia, whereas the non-leukemic strains were comparatively refractory.

There is no evidence that leukemia tended to occur in certain families within the strain. There were no litter mates in this group, nor were there any of common parentage, and only two had a single common grandparent. In other words, although the occurrence of leukemia in this stock seems to have its basis in some inherited factor, inasmuch as this condition has not been observed in heterogeneous or other inbred strains, it is impossible to trace a closer familial relationship within the strain itself. Admittedly, the number of cases observed is insufficient to rule out conclusively such a possibility. About 25 litter mates of the leukemic animals have died, most of them with longer life spans, and none has shown any evidence of the disease. It is true that only 5 animals, 2 males and 3 females, were retained for experimental purposes in each litter. This might have obscured any tendency for the disease to appear in individual families, especially as the disease in this particular group was more prevalent in males than in females. There were 8 males and only 4 females.

In addition to the leukemic rats there was another group of 36 in this series of 365 which showed evidence of marked myeloid meta-

* We wish to acknowledge our indebtedness to Dr. H. C. Sherman, Dr. H. L. Campbell and Dr. L. N. Ellis for making this material available.

plasia and enlargement of the spleen, but no other changes attributable to leukemia. In many the splenic changes simulated leukemia in every respect. The organ measured from 4 to 6 cm. in length. The architecture microscopically was completely obliterated, follicles and venous sinuses being invisible. The pulp was uniformly filled with leukocytes, a large proportion of which were fairly mature, with polymorphic nuclei and neutrophilic granulations in the cytoplasm, readily demonstrated with the Giemsa stain. There were, however, still greater numbers of neutrophilic myelocytes and some eosinophils and megakaryocytes as well. Mitotic figures were numerous.

It is difficult to evaluate this group. It is possible that it includes a few real but very early cases of leukemia, which might have been recognized if white blood cell counts had been available. Possibly the splenic picture represents a preleukemic change. If so, the total incidence of leukemia and potential leukemia is brought up sharply in this strain to 12.2 per cent. It seems more likely that this myeloid transformation is a response to prolonged and widespread pyogenic infection. Females predominated in this group, there being 28. Of these, 23 had extensive uterine infection with the formation of large old abscesses. The entire group showed extensive pulmonary and middle ear suppuration. Evidence of hematopoiesis is almost constantly present in the splenic pulp of this strain of rats. It is not improbable that infection followed by longstanding leukocytosis may have called forth a marked myeloid response in the spleen. It must be admitted that many of the remainder of the animals also showed extensive pyogenic infection of equal severity without, however, any significant alterations in the spleen. No obvious familial relationship, either to each other or to the leukemic animals, was found to exist in the members of this group.

No information concerning the duration of the leukemic process is at hand. The changes in the viscera and the type of cell involved conform in every case to the chronic variety of the disease as it is seen in man. As regards the duration of life, the average age at death of the 4 females was 669 days, as against an average of 801 days for 110 non-leukemic female rats. Three of the 4 died at an age well below that of the average for the female controls. The average life span of the 8 leukemic males was 718 days, only slightly less than the average life span of 100 non-leukemic males, which was 738 days.

Four of the leukemic males died at a younger age and 4 outlived this control average.

It would appear then that the presence of leukemia in these animals did not appreciably alter the duration of life. If anything, they died at a slightly younger age, but 5 of the 12 lived longer than the average of the control groups of the same sex. It will be noticed that the disease was found only in fairly old rats; none was under 18 months of age and the majority were well over 2 years. It is possible that this fact may in part account for the failure to observe leukemia as an incidental finding in the general run of laboratory rats, since the major portion is discarded or sacrificed before reaching the leukemic period.

From the time of weaning all the rats were maintained on diets of known composition. These diets consisted chiefly of ground whole wheat and dried milk powder, in varying proportions, with a few additional constituents such as green vegetables or lean beef. In each instance the diet was of sufficient caloric, protein, mineral and vitamin content to support good growth, nutrition and reproduction. The diets were analyzed to see if any favored the development of the disease. Leukemia developed in animals on six different diets. There was one diet on which 40 animals were fed, none of which developed leukemia. It consisted of: whole wheat 77.44 per cent, whole milk powder 16.41 per cent, butter fat 4.59 per cent, sodium chloride 1.56 per cent. The average life span of the 40 animals was longer for both sexes than that of leukemic ones.

There was a second diet including only 19 animals, 3 of which developed leukemia, an incidence of 15.8 per cent, which is considerably higher than that of any other diet. Its composition was: flour 35 per cent, milk powder 16 per cent, dried beef 10 per cent, lard 7 per cent, whole wheat 6 per cent, butter fat 4 per cent, dried potato 9 per cent, sugar 9 per cent, dried orange juice 4 per cent. The non-leukemic animals on this diet had a shorter average life span for both sexes than the control groups. With the exception of these two discrepancies, both of which are very likely accidental, no dietary influence in the development of leukemia was found. There was certainly no obvious ingredient in the food, the presence or absence of which favored the development of the disease.

The gross features of the disease were as follows: The spleen was

in each case markedly enlarged, measuring from 5.4 to 8.5 cm. in length. In 1 case of chloroleukemia the spleen was tremendous in size, weighing 15.5 gm. and measuring 8.5 by 2.5 by 1.5 cm. It filled more than half of the abdomen. The capsule was usually tense and slightly thickened. The pulp was firm, dark red, non-friable and homogeneous. The lymphoid follicles and trabeculations were indistinguishable. In several cases scattered, irregular, sharply outlined, pale yellow ischemic infarcts had formed. In the chloroleukemia cases the pulp had a slight green cast superimposed on the otherwise grayish red pulp.

The lymph nodes, both deep and superficial, were enlarged from 0.5 to 1.5 cm. across. Their enlargement was somewhat more pronounced in the single case of lymphatic leukemia. In 8 of the cases the nodes were pale yellowish gray and rather firm. In the cervical regions they formed large masses. In the 4 pigmented cases the nodes were a striking, light lime green color.

The liver in some cases was definitely increased in size and grayish red. In the chloroleukemia cases the site of the leukemic infiltrations about the portal areas was more easily recognized since these foci had an unmistakable green hue. The kidneys were almost constantly enlarged, sometimes to three or four times the normal size. They were occasionally mottled with fresh hemorrhages. The enlargement was chiefly at the expense of cortical tissue, the radial markings of which were quite distinct on cross-section. The green coloration was pronounced in the chloroleukemia cases.

The marrow of both flat and long bones seemed abundant and varied from gray to grayish red. In the pigmented cases the entire skeleton was tinted a light green. This was so striking in the flat bones of the skull that it was faintly visible through the scalp. Occasionally other tissues, such as the salivary glands and lungs, showed the presence of green pigment. If abscesses in the lungs and purulent material elsewhere were present, they too had become pigmented in a similar fashion. All the pigmented areas faded slowly on exposure to air and formalin. The color could be intensified by immersion in hydrogen peroxide. After fixation it could be partly restored in the same manner for several weeks, but later it lost this property entirely. Similar phenomena have been described in studies with human chloroma.

The blood clots in the heart chambers and larger veins were usually pale yellowish gray streaked with red. The blood in the cases of chloroleukemia showed no trace of abnormal pigmentation.

Among the incidental findings at autopsy were collections of pus in the auditory bullae. The incidence of middle ear disease at the time of spontaneous death in this strain is almost 100 per cent, although it is rarely found when the animals are sacrificed at a younger age. In 2 cases the foci of suppuration had extended through the floor of the cranial cavity and given rise to brain abscesses. Abscesses in the lungs and in the uterus were occasionally observed. These incidental findings were of the same nature as those found in the non-leukemic animals. If anything they were less extensive, especially in the younger individuals.

Microscopically the leukemic cells were readily identifiable as myeloid in nature in 11 of the cases. They varied in appearance, many appearing to be quite mature and closely resembling polymorphonuclear neutrophils. The nuclei of these cells were segmented or ring-shaped. Other cells appeared less well developed and had spherical or oval nuclei. Neutrophilic granulations were demonstrated with the Giemsa stain. The vast majority of the cells gave a positive oxidase reaction by the Graham method (para-alpha-naphthol-pyronin). Only a few myeloblasts could be identified. There were, however, many mitotic figures and a moderate number of eosinophils and megakaryocytes. In the single case of lymphatic leukemia the cells were of the small round cell type closely resembling mature lymphocytes and having scanty, non-granular, oxidase-negative cytoplasm.

In the bone marrow the leukemic cells had entirely replaced the normal cellular constituents and the adipose cells. The splenic and lymph node architecture was obliterated by massive accumulations of leukemic cells. The lymphoid follicles and trabeculae could not be seen except in the case of lymphatic leukemia. In the spleen of this animal, huge hyperplastic follicles, often partially coalescent, could be made out, but in places small areas of normal pulp intervened. In the liver, foci of leukemic cells were found both in the periportal spaces and in and along the sinusoids. Where this was most marked the liver cells were compressed and atrophic.

Leukemic infiltrations were frequently found in the renal cortex and peripelvic fat. Here they tended to be patchy. In places the

tubules and glomeruli were widely separated by the cellular accumulations. An incidental renal finding present in every case of leukemia and never observed in any of the non-leukemic animals was the presence of large, well defined colloid droplets in the cytoplasm of practically every convoluted tubule. The nuclei of the lining cells were for the most part intact, but the cytoplasm was filled with droplets which varied in size up to that of an erythrocyte. The droplets were particularly conspicuous in Giemsa preparations. They were found in areas where leukemic cells had not infiltrated and can therefore not be considered as a degeneration due to pressure. The nature of this lesion is obscure, but it very definitely appears to be associated with the leukemic process in these animals.

Leukemic infiltrations were also encountered in other organs, such as the lungs, ovaries, uterus, testes, salivary glands, pancreas and heart. The blood vessel contents were rich in leukemic cells.

One interesting finding was the presence of many large phagocytic cells interspersed among the leukemic cells in all 4 cases of chloroleukemia and in 5 of the non-pigmented myeloid leukemia. These cells were large, pale staining elements with a single, small, round, pale staining nucleus situated in no constant place in the cytoplasm of the cells. The cytoplasm was spongy or even foamy in appearance and included coarse nuclear particles which contained no oxidase granules and only rarely gave a faintly positive Prussian blue reaction for iron. With Sudan III they were shown to be filled with fine droplets of stainable fat.

The cells were particularly striking in the bone marrow, lymph nodes and renal and hepatic infiltrations, and less so in the spleen, although fat stains showed them to be present there as well. They appeared to be somewhat more abundant in the chloromatous rats than in the others. Eichhorst [9] has described similar elements in the bone marrow of a case of chloroma. But their absence in all the other reported cases of human chloroma, as well as their presence in the unpigmented cases of rat leukemia, make it seem unlikely that they have any specific connection with the abnormal pigmentation.

It was impossible to detect any histological difference between the non-pigmented and pigmented cases. The leukemic cells in both had the same appearance. Eosinophils and the more mature forms with polymorphic nuclei were not more abundant in the chloroleukemic animals.

A single attempt was made to transmit the disease experimentally. The spleen from 1 of the cases of chloroleukemia where death took place a few hours previously and which showed only slight post-mortem changes was minced in sterile saline and injected intraperitoneally into 12 stock rats. After 8 months the rats were sacrificed and failed to show any evidence of leukemia. Since we have no information concerning the viability of the cells in the inoculum and since the rats used were not of the same strain as the leukemic rat, the negative result is without much significance.

Summary and Conclusions

The spontaneous development of myeloid, chloromyeloid and lymphatic leukemia in a strain of inbred albino rats is described. The total incidence of leukemia in this strain was 3.3 per cent. The disease is one of advanced life occurring chiefly in animals over 2 years of age at death and never in those under the age of 18 months. It is somewhat more common in males than in females. No dietary influence on the development of the disease was observed. The pathological changes are described in detail and conform in most respects to those observed in other species, including man.

One incidental finding not described in the leukemia of other species is the presence of colloid droplets in the convoluted tubules of the kidneys. The nature of this nephrosis and its relation to rat leukemia is unexplained.

Lipoid-containing phagocytes are almost constantly associated with the extravascular accumulations of myeloid cells.

No histological differences between the pigmented and non-pigmented forms of the disease were recognized.

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DESCRIPTION OF PLATES

PLATE 29

FIG. 1. Myeloid leukemia. Perivascular and peribronchial leukemic infiltrations in lung; lumens of vessels filled with leukemic cells.

FIG. 2. Myeloid leukemia. Sternum. Hyperplasia of marrow and infiltration of adjacent skeletal muscle.

FIG. 3. Myeloid leukemia. Infiltration in renal cortex.

FIG. 4. Myeloid leukemia. Infiltration of hepatic sinusoids.

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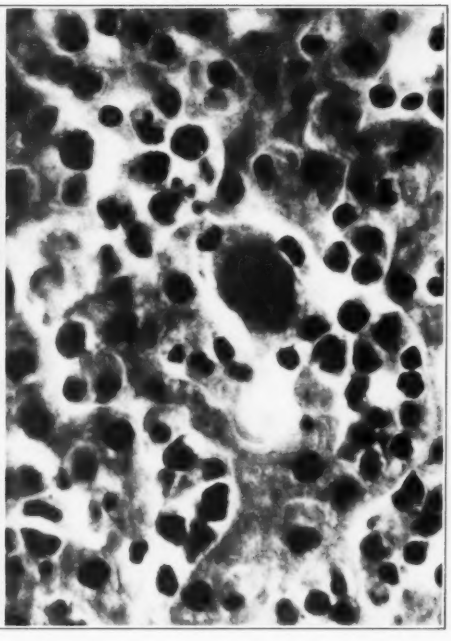

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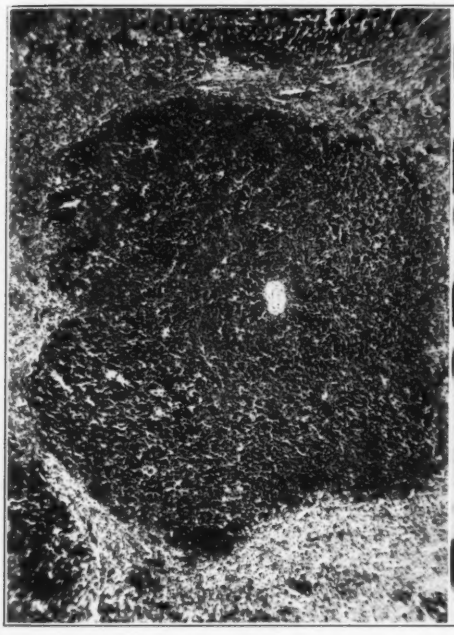
PLATE 30

FIG. 5. Spleen. Lymphatic leukemia. Hyperplasia of malpighian corpuscle of spleen.

FIG. 6. Myeloid leukemia. Cells in the pulp of a lymph node showing irregular nuclear forms.

FIG. 7. Myeloid leukemia. Lipoid-containing phagocytes in lymph node.

FIG. 8. Myeloid leukemia. Colloid droplets in cytoplasm of convoluted tubular epithelium in kidney.



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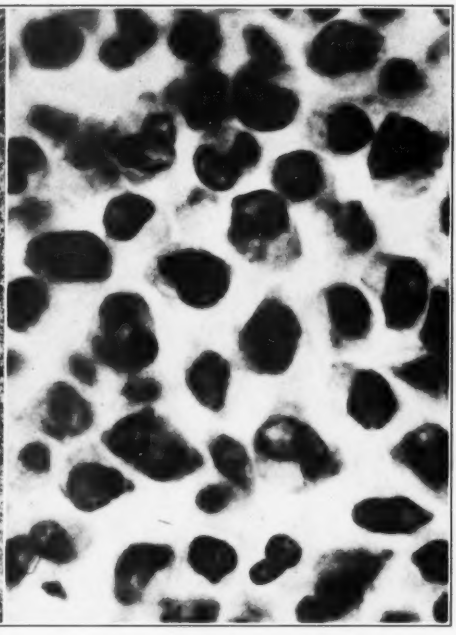

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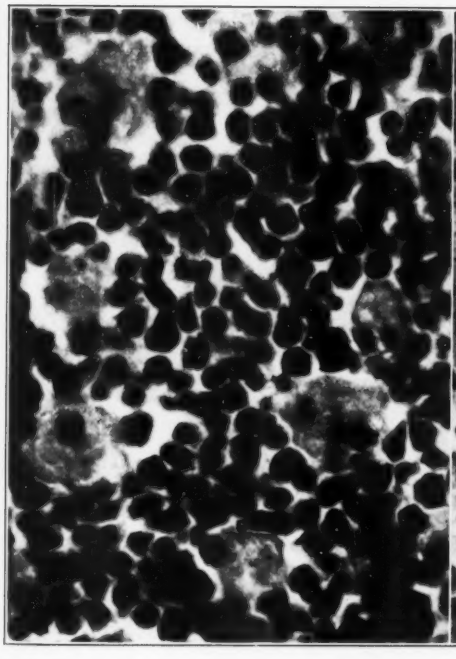

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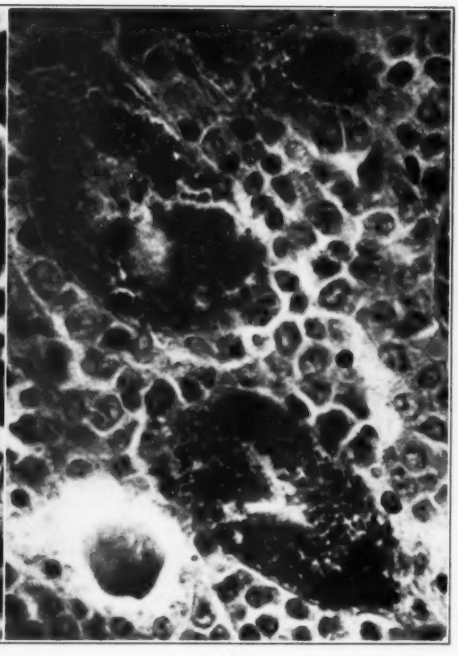

8

Wilens and Sproul Spontaneous Leukemia and Chloroleukemia in Rat

MITRAL STENOSIS WITH INTERAURICULAR INSUFFICIENCY*

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Mitral stenosis with interauricular insufficiency has been an uncommon finding at autopsy in patients who suffered from cardiac disease during life, and only in the past 20 years have definite clinical and pathological facts been correlated in an effort to define a syndrome with salient diagnostic features. Thus, 1 case is here recorded which falls into this classification, and another presenting certain clinical and roentgenological findings which admit of a similar diagnosis is also reported.

Martineau [1] in 1865 was the first to report this condition in the literature. One year later Peacock [2] reported a similar case, and in the next 7 years 3 other cases were added. Firket [3] in 1880 described in detail the clinical and pathological findings in a case of a large auricular septal defect with associated mitral stenosis in a female, 74 years of age, who had survived eleven full term pregnancies. He hypothesized a favorable rôle of the septal defect in that it relieved the circulatory embarrassment ordinarily found in the pulmonary tree in mitral stenosis, believing that the shunting of the blood from left to right auricle, due to pressure differences, conveyed it to the caval veins, thus acting as a safety valve to the encumbered lesser circulation. Later Lutembacher [4] pointed out that such a transmission was effective only when failure of the right auricle had taken place, the main domain of circulatory changes being confined to the pulmonary veins and artery.

Dr. Maude Abbott [5] in 1915 reported a case of a female, 38 years of age, with a history of cardiac symptoms since 14 years of age following an attack of rheumatic fever. The patient had successfully survived one full term pregnancy. Following several breaks in cardiac compensation she died of circulatory failure. Autopsy revealed an old buttonhole type of mitral stenosis with patent foramen ovale 2 by 1.5 cm. in diameter. Both auricles were greatly enlarged, as were the right ventricle and pulmonary artery. The aorta was hypo-

* Received for publication October 14, 1935.

plastic. Clinically the patient had shown a faint thrill and presystolic murmur toward the middle of the precordium in the fourth left interspace, and on two occasions a presystolic murmur at the mitral area with an accentuated second sound. Increased pear-shaped dullness of the heart to percussion was noted. Roentgenograms and fluoroscopic examinations were not made and a positive diagnosis of a septal defect with mitral stenosis was not made during life.

The following year Lutembacher [4] reported a case of a female, 60 years of age, who had survived seven full term pregnancies. She had no history of previous rheumatic fever. On admission she presented all the signs of stasis of the systemic veins. Cyanosis was absent. The heart showed a complete arhythmia but a systolic bruit at the apex preceded by a less intense rumble was determined. In spite of digitalis therapy she shortly succumbed of circulatory failure, exhibiting an intense cyanosis of the lips, cheeks and nose just prior to death. Autopsy revealed an enormous dilatation of the right auricle and ventricle, with considerable muscular hypertrophy. The right ventricle formed the entire anterior aspect of the heart, including its apex. The pulmonary artery was enormously dilated, as were its branches, each one of which was the size of a normal pulmonary artery. The pulmonary orifice was competent, due to the development of the pulmonary valves. Abundant atheromatous plaques were present throughout the pulmonary tree. The aorta was hypoplastic and showed slight atheromatous infiltration. Its valves were undamaged. The mitral valve showed a tight, rigid stenosis, scarcely admitting the tip of the little finger; the tricuspid valve readily admitted four fingers. A large defect in the auricular septum measured 3 by 5 cm.

Drawing conclusions from his case, and others reported in the literature, Lutembacher considered the possibility of the clinical diagnosis and stressed certain anatomical features. Roentgenological examination alone he believed of paramount importance in making the diagnosis, as nothing characteristic could be determined from the cardiac murmurs. Normally in a pure mitral stenosis the outline of the heart is characteristic. The vertical contour of the left border and its pointed appearance bring out at the same time the great development of the left auricle and the relative smallness of the left ventricle. A see-saw-like movement imparted to the heart by the dilatation of this auricle he believed to be more characteristic than

the malformation of the cavity itself. This movement, taking place in a clockwise direction, causes the apex of the left ventricle to indent the diaphragm. The left border of the heart becomes less curved and more nearly parallel with the anterior thoracic wall. At the same time the right border is raised, uncovering the first part of the inferior vena cava. He stressed the fact that when a large auricular septal defect complicates the mitral stenosis the picture is quite different, even disregarding the great dilatation of the pulmonary artery. The greatly dilated right auricle and ventricle give at the same time the appearance of both a transverse heart and a sabot-shaped heart. The movement of its blunted apex is upward and outward in a counterclockwise direction, uncovering all the lower border of the right ventricle normally hidden in the diaphragm. The left contour of the heart takes the form of a broken line. Its superior part obliquely and at the left corresponds to the left ventricle, the right inferior oblique portion to the right ventricle forming its apex.

An interauricular septal defect may be one of several types, depending on the embryological development of the septum primum. In embryos of 6 mm. the septum primum is seen to arise from the dorsal and cranial walls of the atrium, where it grows backward and ventrally to join the endocardial cushions. The primary foramen ovale or interatrial foramen then appears as a narrow aperture between the caudal end of the septum primum and the endocardial cushions. Before these two fuse, a second foramen ovale appears in the dorsal and upper part of the septum primum. The septum secundum appearing in embryos of 9 mm. arises from the ventral and cephalic walls of the atrium just to the right of the septum primum. When the growth of this has caused its dorsal margins to pass beyond the dorsal margin of the secondary foramen ovale, it acts as a flap valve allowing passage of blood from right to left but preventing a reflux. It becomes closed shortly after birth as a result of the raised pressure in the left auricle which approximates the septum primum with the fused left valve of the sinus venosus and septum secundum. Then, when the lower part of the septum primum fails to develop, the defect is termed a persistent ostium primum; when extreme it forms a triloculate heart. A defect in the upper part of the septum is known as a persistent ostium secundum. However, other factors may produce an insufficiency of the auricular septum, such as perforation of the septum by endocarditic processes, various degrees of

reopening, non-adherent or probe-patent flaps forming the secondary foramen ovale by hypertrophy and dilatation of the auricles due to adherent pericardium, or other causes. Clinically all these cases fall into the acyanotic group. The terminal cyanosis often seen is explained by the mixture of venous and arterial blood by the reverse passage of blood from right to left auricle when compensation in the latter has failed.

Dressler and Rösler,[6] reviewing the subject of mitral stenosis with interauricular insufficiency in 1930, reported a case of a female, 30 years of age, in whom the auricular septal defect was discovered shortly after birth. She was free of cardiac symptoms until the age of 22 years when, following an attack of influenza, she failed rapidly. On examination she presented the stigmata often seen with hypoplasia of the aorta, that is, small stature, slender build and signs of delayed puberty. Slight cyanosis of the cheeks and lips was present. X-ray examination suggested an auricular septal defect because of the large pulmonary artery and underdeveloped aorta. Fluoroscopic studies demonstrated the enlarged, pulsating hilar pulmonary vessels. Electrocardiogram showed an enlarged and split R-wave, being up in lead one, and inverted in lead three. The Q R S time was 0.11. The P R interval 0.17. The T-wave was inverted in all three leads. The most conspicuous findings at autopsy were the marked hypertrophy and enlargement of the right heart, associated with a large pulmonary artery and hypoplastic aorta. The left ventricle appeared as an appendage on the posterior surface of the heart, which had been rotated posteriorly because of the greatly enlarged right ventricle which formed both anterior and left borders of the heart as well as the apex.

McGinn and White [7] analyzed 24 cases of mitral stenosis with interauricular insufficiency (not mentioning the 2nd case of Tylecote [8]) reported in the literature up to 1933, including 1 case of their own. Only 2 of these cases occurred in males.

Dr. B. S. Oppenheimer, at the meeting of the New York Pathological Society in April, 1934, presented 8 cases of gigantism of the pulmonary artery, 1 of which showed the syndrome of a large interauricular defect associated with mitral stenosis, bringing the total number up to 26.

Our case, the report of which follows, is the 3rd one reported to have occurred in a male and brings the number up to 27.

Case Report

Case 1. A white male, 67 years of age, was admitted to the medical ward of Saint Luke's Hospital on Dec. 6, 1929, complaining of sore throat, head cold and general malaise. On questioning, he admitted some exertional dyspnea. There was no history of rheumatic fever. Venereal infection was denied.

Examination of the heart showed the left border on percussion outside of the nipple line in the fifth interspace. A thrill and systolic murmur at the apex was transmitted to the axilla. The blood pressure was 104/80. One year later he was readmitted with a diagnosis of auricular fibrillation and acute bronchitis. In 1933 he was twice admitted with cardiac complaints, and blood Wassermanns taken on these occasions were positive. Since his discharge, 5 months prior to his last admission, he constantly suffered from exertional dyspnea, orthopnea and nocturia.

Three days prior to the last admission he began to complain of sore throat, cough and expectoration of frothy, watery sputum. On admission his temperature was 104 F. and he had signs of pneumonia at the base of both lungs. The heart was markedly enlarged both to the right and to the left of the sternum. Systolic and presystolic murmurs were heard at the apex followed by an accentuated first sound. To the left of the sternum in the third and fourth interspace a soft diastolic murmur was heard. The pulmonic second sound was accentuated. The aortic sound was distant. The white blood count was 6800, polymorphonuclears 94 per cent. A portable X-ray was taken of the chest, which confirmed the diagnosis of pneumonia, but the patient was too ill to permit fluoroscopy or further clinical study. Electrocardiograms taken on previous admissions showed normal rhythm, low ST leads one and two, right ventricular preponderance, inverted T in lead three, and notched R in all leads.

The patient succumbed to pneumonia 3 days after admission. An autopsy was performed 7 hours after death and reported as follows.

Postmortem Examination

The body was that of a well developed and well nourished male. Icterus was present, most marked over the upper half of the body. Rigor mortis had set in and postmortem lividity was seen over dependent parts. On opening the thorax no free fluid was found in either pleural cavity. The right lung weighed 1275 gm. Firm old adhesions bound the posterolateral surface of the lower lobe to the parietal pleura. The upper lobe on cut section was dry, crepitant and smooth in its upper half, its lower half being irregularly congested. The middle and lower lobes showed a confluent type of bronchopneumonia. The left lung weighed 725 gm. Old adhesions were present over the upper and lateral surface of the lower lobe binding it to the parietal pleura. The lower lobe showed a pneumonic infiltration similar in character to that found in the right lung. The pulmonary artery was markedly dilated in its ramifications throughout the lung tissue. The heart weighed 700 gm. The peri-

cardial sac contained 130 cc. of bile-stained fluid. The transverse diameter of the heart was greatly enlarged, the lateral border of the right auricle extending to the midclavicular line at the level of the fourth rib. The right ventricle formed the greater portion of the anterior surface of the heart and the apex reached the sixth left rib in the anterior axillary line. Large, irregular, white fibrous plaques were present over both the right auricle and the ventricle. The right ventricular wall measured from 15 to 17 mm. in diameter at the base. The left ventricular wall measured 2 cm. in diameter. On opening the mitral valve its leaflets were found thickened, sclerotic and bile-stained. It measured 9 cm. in diameter. The free edge of the anterior flap was more thickened than the posterior valve edge. The chordae tendineae showed some fibrosis and shortening. An oval auricular defect just above the septal flap of the tricuspid valve and below the mouth of the auricular orifice measured 3 cm. in diameter. Its location corresponded to an arrest in the development of the ostium primum or primary interatrial foramen, the secondary foramen ovale being closed and showing no defect. The coronary orifice was dilated, measuring 12 mm. in diameter. The vein was similarly enlarged. The aortic valve measured 6 cm. in diameter. Garland-like, fine verrucae extended down from both sides of the left posterior commissure, 3 mm. below the free edge of the cusps. The valve commissures were fused, but the valve leaflets were quite pliable. The coronary vessels showed a moderate amount of sclerosis and tortuosity. The thoracic aorta measured 6.5 cm. in circumference throughout its extent and was remarkably free from scarring or atheroma. The tricuspid valve measured 14 cm. in diameter, and its flaps were mobile but somewhat sclerotic. The pulmonary valve measured 11.5 cm. and its cusps were sufficient. The pulmonary artery was of a similar circumference to its point of bifurcation. Its wall showed no gross changes.

On microscopic section the aorta showed slight degenerative changes, but no evidence of inflammatory or specific lesion. The pulmonary artery showed slight fraying of the elastic tissue in its media with infiltration of the muscle cells with calcium salts, but no other remarkable changes.

CASE 2. A 2nd case came under our observation presenting X-ray and fluoroscopic signs suggesting mitral stenosis with interauricular insufficiency. The patient was a white male, 41 years of age, who was first admitted on Jan. 7, 1933,

complaining of an attack of dyspnea and palpitation of 4 hours duration. One year previously after a sudden exertion the patient became dyspneic and dizzy and complained of palpitation lasting from 3 to 4 hours. This subsided with rest in bed. Since this attack he suffered from mild attacks of dyspnea and palpitation, always following exertion or excitement. Exacerbation of these symptoms caused him to seek relief at a hospital. He gave no history of rheumatic fever.

Physical examination showed a slightly emaciated, pale white male of asthenic habitus. Paroxysmal tachycardia with ventricular systoles was present. On restoration of normal rhythm, which occurred during examination, the mitral first sound at the apex was partially replaced by a low pitched blow. A faint, light diastolic murmur was heard at the apex. The apical second sound was loud. Systolic blood pressure was 90. The pulmonic second sound was accentuated. Electrocardiogram showed normal rhythm with right ventricular preponderance, high ST in lead one, low ST in leads two and three, low T in lead one, inverted T in lead three, notched R in all leads. X-ray examination showed a moderately enlarged heart with a bulge in the region of the left auricle which overshadowed the aortic region. Considerable thickening of both lung roots was present. Re-examination in the lateral position failed to reveal any bulging of the heart chambers. Fluoroscopic studies were not made at this time. The patient improved with rest and digitalis, and was discharged 3 weeks after admission.

He was in fair health until 4 months prior to his present admission, when he suffered from chest cold, fever, malaise, a non-productive cough and frequent exacerbation of his exertional complaint. The heart was moderately enlarged to the left. The first sound at the apex was faint; the second sound had a rapid reduplication both at the apex and the base. Soft, blowing, systolic murmurs were heard at both the pulmonic and the aortic areas. The pulmonic second sound was increased. Blood pressure was 98/65. Electrocardiogram showed a prominent P wave in lead two but otherwise essentially the same as the previous examination. X-ray retakes showed a mitral configuration of the heart with a greatly enlarged pulmonary artery and a hypoplastic aortic knob. The right auricle was dilated, its contour rounded, particularly at its lower border where it formed an acute angle with the diaphragm. Fluoroscopic examination showed the enlarged shadow of the pulmonary artery to give a strong expansile pulsation.

The patient is at present well and under observation at the cardiac clinic.

Summary and Conclusions

A brief review of several outstanding contributions to the study of mitral stenosis with interauricular insufficiency collected from 26 cases reported in the literature up to the present time is offered and 1 case of our own is added.

The pathogenesis of the septal defect concerns the developmental arrest of the primary or secondary foramen ovale or the reopening of a probe-patent secondary foramen by an acquired hypertrophy and dilatation of the auricles. Perforation by endocarditic processes is rare.

The mitral stenosis is an acquired, not a congenital, lesion, although only 3 cases gave a history of rheumatic fever and one had several attacks of chorea.

The syndrome is rare in males, only 3 cases including our own being reported.

The stigmata associated with hypoplasia of the aorta, that is, slender build, small stature and delayed puberty, may or may not be present. The symptoms most frequently complained of are exertional dyspnea, palpitation and easy fatiguability. Cyanosis is absent, or present only as a terminal event. Club fingers were reported in only 1 case. Auscultatory signs are variable and may be absent. An apical systolic murmur is most frequently heard, being present in 16 cases. It was accompanied by a diastolic murmur maximal at the apex in 9 cases. An apical thrill was present in 5 cases. The pulmonic second sound is usually accentuated. Three cases had electrocardiograms which showed a right ventricular preponderance and notched R-waves in all leads. P-waves showed no distinct abnormalities. All the hearts were enlarged, due mainly to dilatation and hypertrophy of the right auricle and ventricle. The left auricle was enlarged, but did not attain the size of the right auricle. The left ventricle was usually small and rotated posteriorly, unless there was a complicating aortic valve lesion. Dilatation of the pulmonary artery with a small aorta was present in 16 cases.

Roentgen examination demonstrates these anatomical points and is of paramount importance in making the diagnosis. Fluoroscopy shows the right ventricle to form the greater part of the anterior surface of the heart and its apex, imparting an upward and outward movement to the latter. There is an expansile pulsation of the pulmonary vessels at the hilum.

The prognosis simulates somewhat that of mitral disease. In 13 of the 27 cases death occurred at 30 years of age or under, the average age at death being about 35 years. Females have been noted to survive several pregnancies with this condition.

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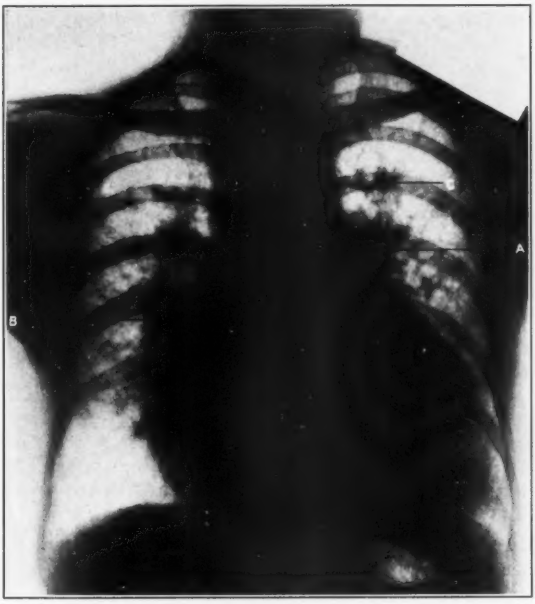
DESCRIPTION OF PLATES

PLATE 31

FIG. 1. Chest X-ray plate in anteroposterior diameter. Large transverse diameter of heart, small aortic knob (C). Large pulmonary conus (A) and prominent right hilar shadow (B) — pulsating pulmonary artery. Prominent contour of rotated, dilated right auricle.

FIG. 2. Anterior view showing position of heart as it appeared in the thoracic cavity. The right-sided hypertrophy rotating the left ventricle (A) posteriorly and the disproportion between the pulmonary artery (B) and aorta (C) are apparent.

FIG. 3. Heart opened from left side. Large auricular septal defect (A) and thickened, contracted mitral cusps (B) visible. Enlarged branch of pulmonary artery (C) and its relations to the aorta (D) can be seen.



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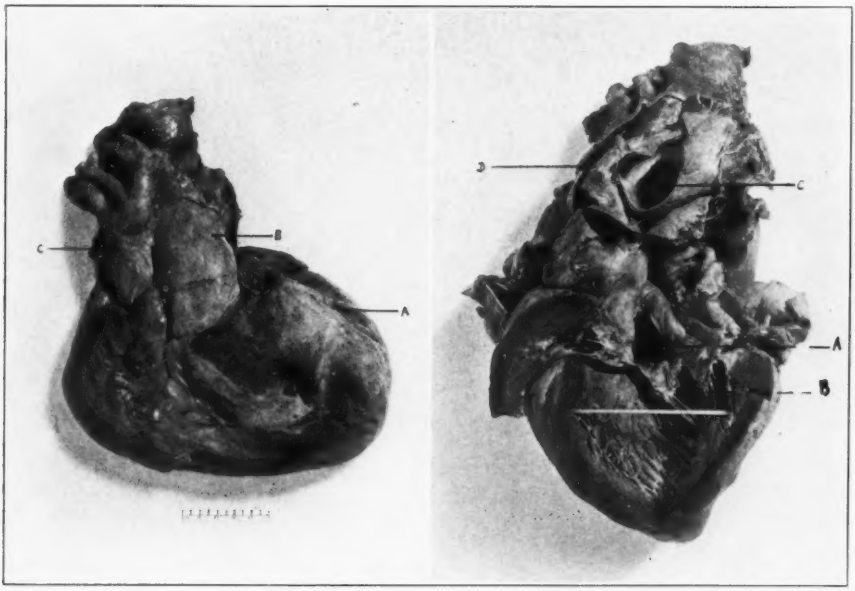


2 3

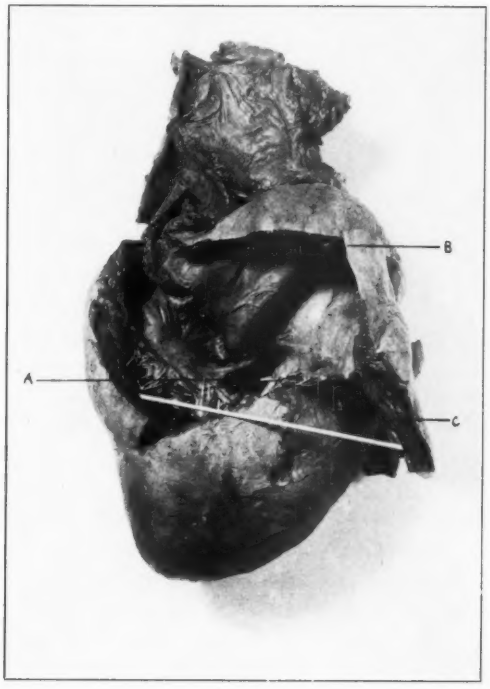
Sailer Mitral Stenosis with Interauricular Insufficiency

PLATE 32

FIG. 4. Right side of heart. Auricular defect (A) just above membranous portion of interventricular septum. Right auricle (B) greatly dilated and hypertrophied. Right ventricular wall (C) hypertrophied.

FIG. 5. Chest X-ray plate in anteroposterior diameter. Greatly enlarged pulmonary artery (A) and inconspicuous aortic knob (B). Enlarged hilar shadows (C) are branches of the dilated pulmonary artery which are seen to pulsate on fluoroscopic examination.

FIG. 6. Lateral view of same patient showing relative proportion of enlarged pulmonary artery (A) and small aorta (B).



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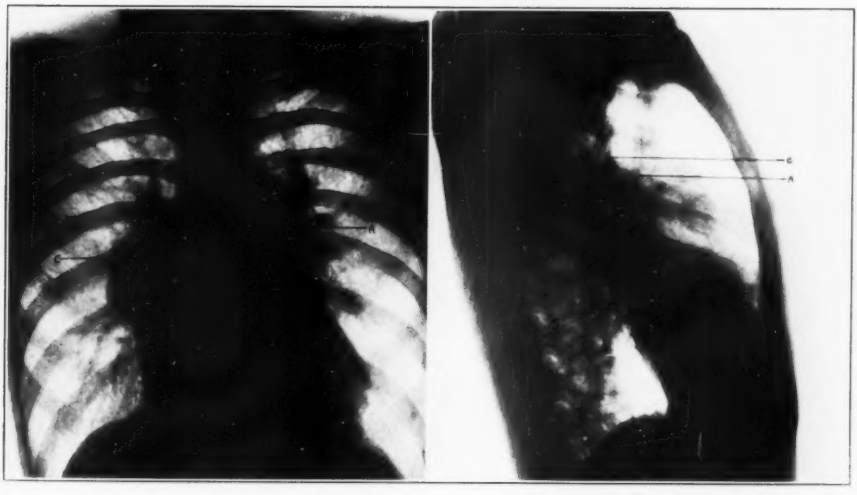

5 6

THE BRAINS OF INFANTS AND CHILDREN IN RELATION TO POSTMORTEM TIME, TOXICITY AND CONVULSIVE STATE *

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Aside from the well defined nervous diseases of childhood, neuropathology has occupied itself chiefly with the study of adult brains. One of the first exceptions was the examination made by Brown and Symmers [1] of the brains in 8 children suffering from an unusual syndrome of convulsions, diarrhea and fever. Since they found an edema of the ganglion cells and of the endothelium of the blood vessels, they concluded that the cases were serous encephalitis. Following this, Grinker and Stone [2] described the neuropathological findings in 10 children suffering from acute infections such as bronchopneumonia, septicemia and scarlet fever, complicated by various nervous manifestations. Their chief observations were swelling and chromatolysis of the ganglion cells, neuronophagia, and an increase in the cytoplasmic glia and endothelium. They believed that these changes were evidences of toxic encephalitis. Under a similar title Low [3] presented the cellular reactions in the brains of 5 children with endocarditis, colitis and bronchopneumonia associated with convulsions, coma and rigidity. He divided his cases into two groups: (1) those in which the ganglion cells were liquefied, the peracute stage; and (2) the acute stage, where vacuoles were present in the ganglion cells. Regressive glial changes accompanied the former and progressive ones the latter.

Material and Results

The present examination of 34 children's brains has been undertaken to determine what may be considered normal and what pathological alterations. The ages of the children were from 12 days to 4 years and the diseases the usual ones of childhood. From most of the brains blocks had been taken and fixed in alcohol. Other brains were fixed *in toto* in formalin and blocks removed after 2 weeks. In some cases the brains remained longer in formalin, but not more

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than a month or 6 weeks, since too long fixation causes cell shrinkage and difficulty in staining. In all the formalin-fixed brains the blocks were washed for 2 days in running water before dehydration. Also, the cases were limited to those autopsied within 15 hours of death, as a longer period of time produces an architectonical distortion from a softened cortex, which is further complicated by the enlarged spaces about the blood vessels. The cytoplasm of the upper cortical ganglion cells is, in general, completely destroyed, while it varies in amount in the lower cells. All the cells are thus apparently decreased in size. The cytoplasm may take a deep stain with pseudo-Nissl substance sharply contrasting with the almost invisible glia, or the glial nuclei may appear dark, small, beaded and not unlike those of the ganglion cells with a poorly staining nucleolus. In other cases the scanty cytoplasm and nuclei may seem fairly well stained, but the nuclei are so much alike that the different types of cells are not easily distinguished except for the larger ganglion cells. In the basal ganglia the cells are shrunken and the cytoplasm may be pale or deeply stained with a usually dull, polymorphous nucleus. Within the confines thus established the similarities and variations in the appearance of the ganglion cells allowed the formation of three groups.

GROUP 1. By the thionin blue stain, the cortical ganglion cells are poorly and irregularly stained, varying from pale pink or blue to dark blue. In the formalin-fixed material the cytoplasm and nuclei are darker but less distinct in the bluish tinged nerve tissue than in the alcohol-fixed material. The barely discernible outline of most of the cells appears to give them a normal size, but the decreased cytoplasm suggests a poorly developed cell. Many ganglion cells in the upper cortical layers have no cytoplasm. That of others is thin and granular, often limited to small particles at the border of the cell or to a narrow strip around the fairly large nucleus. The latter generally stain like the cytoplasm, but some are opaque and violet-hued. Narrow ganglion cells with a deeply stained cytoplasm and nucleus are also numerous. Figures 1 and 2 present a fairly typical picture of the disintegrated and poorly stained cytoplasm in the upper cortical cells. In the lower cortical layers the entire cytoplasm of the larger cells is frequently present. The ganglion cells of the cerebellum, the basal ganglia, the pons and the medulla oblongata are moderately dark blue or pink with a fair abundance

of cytoplasm and some Nissl substance in the alcohol-fixed material but little or none in the formalin-fixed. Central chromatolysis is found in a few scattered cells of the basal ganglia. The cytoplasm of the glia is seldom visible. In the oligodendroglia the nuclei are small and deep blue, while those of the astrocytes are large and vary from light blue to dark, the latter in cases of longest postmortem time and fixation in formalin. Few microglia are stained, the nuclei taking a paler blue stain than the oligodendroglia. The vessels are somewhat pulled away from the nervous tissue, the space being more pronounced when the postmortem time is prolonged. Perivascular cells and glial reaction are present in the cases of meningitis, together with demyelination in occasional foci of acute inflammation. Microglial arborization is distributed along the capillaries in the molecular layer of the cerebellum in the cases of tubercular and acute meningitis.

Cases of this Group: (*Division A.*) Ages: 12, 14, 15, 17, 24 and 35 days. Autopsy time: 12, 6, 6, 2, 10 and 12 hours postmortem. Cases: cerebral hemorrhages and softening in a convulsive state of 9 days duration; dermatitis; hydrocephalus with small hemorrhages and softening in the white substance together with many ischemic cells in the lower cortex; septicemia; Mongolian idiocy with malformation of the heart; and intestinal toxemia with delayed cerebral development.

(*Division B.*) Age: 2 months. Autopsy time: 8, 12 and 12 hours postmortem. Cases: intestinal toxemia; cellulitis; and bronchopneumonia.

(*Division C.*) Ages: 7, 8, 10, 10½, 12, 18, 30 and 36 months. Autopsy time: first case 10 hours, the others 12 hours postmortem. Cases: pyelonephritis; tubercular meningitis; acute meningitis (2); bronchopneumonia; hemorrhagic purpura of 2 days duration; oxycephaly, with malignant tumor of one kidney and bronchopneumonia; and cellulitis. Convulsions were present in the cases of meningitis and hemorrhagic purpura. The ninth case was a child of 6 months who had suffered for several weeks with diarrhea and secondary anemia. A second blood transfusion, given at a week's interval from the first, was followed by convulsions and death in an hour. Autopsy time: 15 hours postmortem. The ganglion cells are like those of the group except for the many ischemic ones, which are found either isolated or in small bleached foci. The accompanying

glia are unstained or pale. Aside from these areas and apparently independent of them, the macroglia of both the cortex and the white substance show a cytoplasmic increase and the microglia are increased in numbers, their bodies frequently swollen and the processes lost. This reaction of the glia, shown in Figure 3, is most pronounced in the lower cortical layers. A perivascular demyelination seems to have occurred in the white substance, although the usual fat deposit in the perivascular spaces is not increased.

GROUP 2. The outline of the cortical ganglion cells is sharper than in the previous group and more distinct in the alcohol-fixed material than in the formalin-fixed. A slight pink or bluish tinge is given the nerve tissue. Many cells of the upper cortical layers and most of those of the lower are filled with a faintly pink or dark violet granular cytoplasm in which Nissl substance is not visible. Other ganglion cells have very little cytoplasm and a rather punched-out appearance, as in Group 1. A few cells are small and dark. The nuclei of the ganglion cells are large and stain like the cytoplasm, occasionally lighter or darker. In other parts of the brain the cytoplasm fills the cells and is deep pink or blue, showing Nissl substance in most cells. The nuclei of the glia are of fair size and less deeply stained than in the first group, but the cytoplasm is shadowy. Most of the vessels are not detached from the nervous tissue. In the case of meningitis the same hyperplastic glial changes are present as have been described in the first cases, but the microglial cytoplasm is better stained; microglial arborization is, also, a pronounced feature in the molecular layer of the cerebellum.

Cases of this Group: (*Division A.*) Ages: 2 and 3½ months. Autopsy time: 1½ and 3½ hours postmortem. The first case was one of amyotonia congenita beginning in the 2nd week of life. Later the child had to be tube fed and died of bronchopneumonia. Aside from the description of the cells already given, characteristic central chromatolysis of the ganglion cells is found in the nuclei of the pons, the medulla oblongata and of the basal ganglia. Many of these cells are undergoing neuronophagia by microglia.

The second case was a previously healthy child of 3½ months in whom an attack of vomiting preceded the onset of successive convulsions and cyanosis. The temperature rose to 104° F., where it remained until death on the 2nd day. The only pathological changes noted at autopsy were otitis media and congestion of the

lungs. Ischemic ganglion cells are prominent, though isolated, in the cortex. Developmental retardations are also observed. The vessels are dilated with a few agonal perivascular red cells. In the molecular layer of the cerebellum are extensive foci of microglial arborization.

(*Division B.*) Ages: 4½ months to 2 years. Autopsy time: 5½ to 8 hours postmortem. The clinical histories were those of intestinal toxemia; malnutrition with bronchopneumonia; acute meningitis with cortical ischemic cells; and whooping cough of 2 months duration, ending in a temperature of 105° F. for 5 days and bronchopneumonia. The following cases are unusual. One was a child of 11 months with whooping cough of several weeks duration. Frequent convulsions and a temperature of 103°–105° F. developed in the 2 days previous to death. Necrobiosis or bleaching of all but a few cells occurred in both cornua ammonis, one of which is seen in Figure 4. Extensive bleaching of the cortex and the marginal white substance may be observed adjacent to the cornu ammonis in the same figure. Large and small areas throughout the cortex are similarly affected. The ganglion cells in these areas are chiefly ischemic ones, triangular shadow forms with homogeneous, pyknotic, triangular nuclei. Other cells are pale with normal appearing nuclei. The glia are few, showing either a pale or a pyknotic nucleus. Fairly well stained ganglion cells of this group are still present in the vicinity of the larger vessels and constitute a characteristic finding of the necrobiotic regions. In the apparently unaffected cortex surrounding the bleached areas are numerous ganglion cells of irregular shape. Their dark distorted nuclei in more or less pale cytoplasm proclaim them ischemic cells, less completely destroyed than the shadow forms. Occasionally, nuclear bits are present in and outside of the cytoplasm. The neighboring glia have also been affected, being either absent or staining poorly. Agonal hemorrhages are seen in the cortex and the vessels are dilated.

The sixth case, a child of 8½ months, had a cough (whooping cough?) for 1 week. Sudden convulsions and cyanosis occurred with death in 24 hours. The Sommer sector of one cornu ammonis is entirely bleached. Microglial arborization is marked in the molecular layer of the cerebellum. An occasional ischemic cell is found in the cortex.

The seventh case was a previously healthy child of 8 months who became ill with a rash followed on the 2nd day by a convulsion of

1½ hours duration. The temperature rose to 104° F. On admission to the hospital the convulsions became continuous. The disease terminated fatally on the 4th day with a temperature of 107° F. The blood vessels of the cortex and white substance are markedly dilated. Numerous cortical ganglion cells and those of the Sommer sector of one cornu ammonis are ischemic. The entire cornu ammonis of the opposite side is necrobiotic. In Figure 5 are a few ischemic ganglion cells of this destroyed area. Widely distributed microglial arborization is found in the molecular layer of the cerebellum. Ischemic ganglion cells and pallor of the nerve tissue are present in the thalamus and head of the nucleus caudatus. The cells are less triangular than those of the cornu ammonis but the nuclei are similar.

GROUP 3. Autopsy time: 1 to 4 hours. Ages: 7 months to 4 years. The majority of the cortical ganglion cells of this group are filled with a smooth or finely granular cytoplasm staining a distinct blue in formalin-fixed material or a deep bluish pink in alcohol-fixed material. The cells are clearly dissociated from the surrounding glia and unstained nervous tissue. A small amount of Nissl substance is found in a few lower cortical cells in the case of 7 months, while a larger amount is present in more of the same cells in the older cases. All the nuclei are large and stain somewhat paler than the cytoplasm, though distinct. Figures 6 and 7 illustrate the better preservation and staining of the ganglion cells in this group than in the first one. The cytoplasm of the oligodendroglia and microglia is either pale pink or bluish in color; that of the macrocytes is colorless or pale pink. The nuclei of the latter are very light blue; those of the oligodendroglia are much smaller and moderately dark blue in color, while the nuclei of the microglia vary in size, shape and depth of bluish stain. The vessels are not separated from the nervous tissue. The cells of the medulla oblongata, the cerebellum, the pons and of the basal ganglia are filled with distinctly stained cytoplasm and Nissl substance.

Cases of this Group: Acute peritonitis and otitis media of 5 days duration with a temperature of 105° F. in a child of 20 months; second degree burns with severe shock and death in 24 hours, age 4 years; whooping cough of 5 weeks duration with fairly numerous convulsions during the last week, bronchopneumonia, and a terminal temperature of 109° F., age 7 months. The last 2 cases have

small agonal hemorrhages in the cortex. The fourth case was one of lead poisoning of 5 months duration in a child of 2 years. Frequent convulsions, rigid neck and uncertain gait were the chief symptoms in the first few months. Under treatment the child improved, but suddenly died in coma. The meninges are slightly thickened. The astrocytes in the gray and white substance are increased in size and number, as are also the microglia. The oligodendroglia show little change in size or frequency. In many areas the spaces between the cortical ganglion cells appear to be widened and partially filled with glia. Small agonal hemorrhages are found here and there in the cortex. Many Purkinje cells are missing in the cerebellum, while numerous foci of microglial arborization with dark blue-staining deposits are present in the molecular layer. The ganglion cells of the Sommer sector and in the end leaf of one cornu ammonis are entirely replaced by glia.

Experimental

Time Postmortem

Three 3 months old healthy kittens were killed with ether and autopsied under various conditions. One was immediately autopsied; one-half the brain was placed in 95 per cent alcohol, the other half in formalin. The second kitten was left at room temperature for 12 hours and the third was kept in the icebox for 12 hours. Both were then autopsied and the brains fixed as in the first kitten. The ganglion cells of the first kitten are sharply stained against a clear background, with Nissl substance in the larger cells. The glial cytoplasm is faintly stained and the nuclei of good size and color. Slight shrinkage of the ganglion cell cytoplasm occurs in the formalin-fixed material. In the second and third kittens the cytoplasm in many ganglion cells appears pale, thin and punched out, particularly in the frontal and occipital poles. The nuclei vary in shape and tend towards a darker tint than the cytoplasm. Many cells are narrow with deeply stained cytoplasm and nuclei. Figures 8 and 9 show the normal cell and its postmortem dissolution. Other findings in the second and third kittens are a bluish stained nervous tissue and a widened space between it and the blood vessels. The glial nuclei are small and deeply stained, more marked in the formalin-fixed material and in the kitten left at room temperature. The cells of

the basal ganglia, the pons and cerebellum are fairly well stained with Nissl substance in the larger cells. In the cord the anterior horn cells contain vacuoles. Perivascular hemorrhages are present in the cortex and white substance.

Carbon Tetrachloride Poisoning

Three adult healthy rabbits were exposed to the fumes of carbon tetrachloride. One died from overdosage at the first administration. The other two were placed in an airtight box with 100 cc. of the fluid. After a few minutes a twitching of the nose and shaking of the feet occurred. These signs of irritation were followed at varying intervals by gasping for air, general or localized convulsions and, lastly, by complete anesthesia. Twice daily the rabbits remained in the box from 20 to 50 minutes, depending upon the rapidity of their reaction. One rabbit, after 6 days of the experiment, was not removed until after it had been exposed for 1 hour to the drug, since no response had been obtained. This animal died in the night from convulsions. The third rabbit was killed with ether after 10 days, when it showed some loss of weight and was less easy to arouse from the tetrachloride. The cortical cells are well stained in the first and third rabbits. The glia cells do not appear to be increased in number or size. The second rabbit, autopsied more than 12 hours after death, shows deeply stained, small nuclei in all cells and marked cytoplasmic loss in the ganglion cells in all parts of the brain. The nervous tissue is also bluish and wide spaces are present about the vessels. The latter contain many postmortem bacteria. An increase in the glia is not observed.

Discussion

The loss of cytoplasm in the ganglion cells of the first group and in normal kittens autopsied after 12 hours has been called liquefaction by Low.[3] He does not consider this a postmortem change but a toxic one, since the oligodendroglia he found within the border of the liquefied cells appear to denote neuronophagia. Oligodendroglia are frequently found within well preserved ganglion cells, and like those in the postmortem autolyzed cell of the normal kitten in Figure 9 seem to indicate an overlapping of cells rather than neuronophagia. If true neuronophagia were taking place, then numerous microglia would be found in and about the cells. Low further doubts that a

selective postmortem influence could produce liquefied cells only in the cortex, since he did not find them in the basal ganglia. As liquefaction, or autolysis (the preferred designation) did not appear in the cells of the basal ganglia of Group 1 or of the late autopsied kittens, it is suggested that postmortem changes may be hastened by bacteria which should find the large vascular bed of the cortex a more fertile ground than the basal ganglia. Also, when the time between death and autopsy is prolonged, the same autolysis occurs in the basal ganglia cells. Chromatolysis of the cells was stressed by Grinker and Stone [2] as characteristic of toxic reaction. Some chromatolysis occurred in the brains of shortest postmortem time, but it was chiefly demonstrable in the cortical ganglion cells of long postmortem time. As the Nissl substance can be stained at the 7th day in the basal ganglia and in some cells of the cornu ammonis, but not until the 7th month in the cortical cells of this series, its absence appears less a developmental process than a susceptibility to postmortem influences. The central chromatolysis in the basal ganglia cells was so frequent that it is not considered of pathological importance.

It may seem that the dissolution of the cytoplasm in the cortical cells in the first group is evidence of a toxic destruction. However, the diversity of the diseases in this group offers no common factor as the source of toxicity. While the ninth case offers some difficulty, because it is clearly one of toxicity, no apparent relation exists between it and the other cases of the same group. On the other hand, the cases of meningitis, hemorrhagic purpura and intestinal toxemia do not appear distinct from similar cases in the other two groups but they are separated by greatly dissimilar cells. It is obvious that the ganglion cells in different brains may suffer individual reactions to toxicity, but there must be some equality between the appearance of the ganglion cell and the clinical history, before the density of the cytoplasm and stain can be regarded as proof of toxicity. Scherer [4] does not consider the ganglion cell particularly sensitive, since normal ones may be found in vascular lesions, in areas of multiple sclerosis, in gliomas, and in parts affected by Wilson's disease. Certainly the toxicity of the cases of lead poisoning and second degree burns cannot be questioned, and yet the ganglion cells show little of the cytoplasmic disintegration and pallor of the first group. Nor is the difference in the appearance of the cells in the cases of whooping

cough in the second and third group explainable on the basis of toxicity, as the symptoms appear to be of like severity. Only the autopsy time seems to unite and divide the cases. The loss of cytoplasm, the poor and irregular staining of the cells, the large perivascular spaces, hyperchromatic glial nuclei and the tinged nerve tissue found in the late autopsied kittens are undoubtedly postmortem changes and similar to the findings in the first group, a similarity which supports the formation of the groups according to the postmortem time.

The exceptions to the groups are the youngest cases, as they are all in Group 1 regardless of the autopsy time. The earliest recognizable influence of the autopsy time on the cortical ganglion cells occurs at 2 months in this series, since the case of amyotonia congenita, autopsied at 1½ hours, is placed in Group 2, while other cases of the same age, but longer postmortem time, are in Group 1. This disease affected so many of the cells in the cord, the thalamus and the nuclei of the pons and medulla oblongata, that the cortical ganglion cells might be expected to resemble those of Group 1, if this group really represented pathologically changed cells. On the other hand, the cortical cells are not as well stained as in older cases of the same or longer postmortem time. But since it is not possible to claim a more pronounced toxicity for the case of amyotonia congenita than for those in Group 3, or to recognize its similarity to all those in Group 2, it may be concluded that the difficulty in obtaining well stained cortical cells in the youngest infants tends to confirm the contention of Spielmeyer [5] that the brains of infants undergo rapid postmortem dissolution. The explanation for this may be found in the high water content of the brain at birth and in the chemical changes which occur during the first 6 months of life.[6] The determining factor of age in regard to postmortem dissolution ends at 7 months in this series, as the cells in the case of whooping cough with convulsions and high fever are similar to those of Group 3.

The growth of the microglia and of the oligodendroglia, the increased size of the macroglia, together with the demyelination in the foci of inflammatory cells, are the expected reactions to infection in the cases of meningitis. The hyperplasia of the macroglia and of the microglia in the cases of lead poisoning and death following a second blood transfusion not only occurred independently of the ganglion cells (1 case is in Group 1, the other is in Group 3) but was so dis-

tinct and so pronounced in both the gray and white substances as to indicate that the important elements in toxicity are the macroglia and the microglia. Although the oligodendroglia must undergo some change in toxicity, their rôle is not easily demonstrated among the flamboyant forms of the astrocytes and microglia. The absence of glial reaction in the other cases of allergy suggests that the blood transfusion case was one of longer toxicity than the symptoms indicate, perhaps beginning at the first transfusion. Glial changes were not present in the rabbits subjected to carbon tetrachloride; this may mean either that the drug does not produce the type of irritation needed to affect them, or that the experiments were terminated before a glial response could take place. The glial findings of Biancalani [7] could not, therefore, be confirmed, although he obtained the same nervous symptoms during similar brief exposures to carbon tetrachloride. The "liquefied" ganglion cells in his rabbits are identical replicas of the postmortem autolyzed cells in the kittens and rabbit of this study.

It is evident that the most important lesions in this series are the bleached areas of the cornu ammonis, the scattered ischemic ganglion and glia cells, the microglial arborization in the molecular layer of the cerebellum, the ischemic cells of the thalamus and nucleus caudatus and the bleached areas of the cortex, all related to the convulsive state. Since Purkinje cells were not missing in these cases and the disturbed cerebral areas were free of the mesodermal activity which shortly follows injury,[8] death must have succeeded the cerebellar and cerebral changes by only a brief interval. In the case of lead poisoning, the glial scar of one cornu ammonis, the glial lawns of the cortex, and the loss of Purkinje cells are evidences of old injury produced by the convulsions which occurred several months previous to death. As epilepsy has been shown to cause bleaching of the cornu ammonis and microglial arborization of the cerebellar molecular layer, Spielmeyer [9] believed that these areas, because of their poor blood supply, are especially vulnerable to the vascular contractions which sometimes occur in convulsions. The closely interwoven vascular net of the cortex demanded another explanation of its bleaching, and for this, Spielmeyer [10] proposed the hypothesis of localized vascular spasms. Recent experiments [11, 12, 13] in regard to the vascular status in convulsions have neither proved nor disproved Spielmeyer's theory of necrobiosis. The preservation of the ganglion cells

around the larger vessels in the bleached areas, as can be seen in Figure 4, seems to indicate that the smaller vessels and capillaries are the worse sufferers. The widespread ischemic cells in the cortex and in the basal ganglia, the latter a less frequently noted change, suggest not so much a localized spasm as a nervous disturbance of the entire capillary bed. The dilated vessels may mean either a rebound from contraction or a long dilatation which could produce the same lesions as a contraction. They may also have caused the perivascular hemorrhages of the cortex either before or during the time of death.

Summary and Conclusions

A study of 34 infants' and young children's brains from cases of acute infections, second degree burns, allergy and lead poisoning did not show changes in the ganglion cells that could be attributed to toxicity. The loss of cytoplasm and the density of its stain, the tingeing of the nerve tissue, the hyperchromatosis of the nuclei and the perivascular spaces varied somewhat with age and the fixative, but chiefly with the postmortem time. The indications of toxic injury were found rather in the reactions of the microglia and the macroglia.

The most severe and frequent lesions in the brains were those produced by the vascular disturbances of the convulsive state which accompany many diseases of infancy and young childhood.

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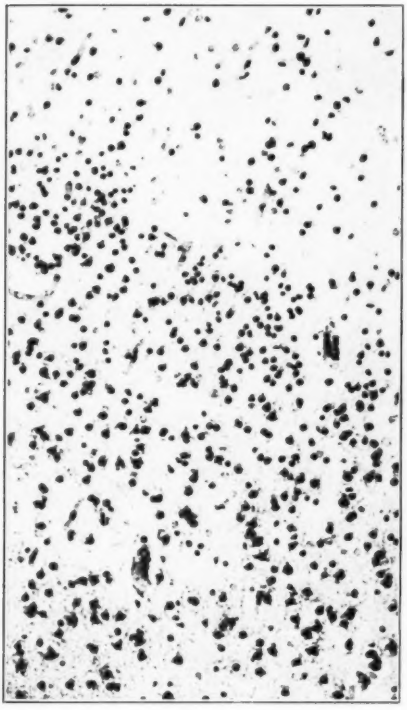
DESCRIPTION OF PLATES

PLATE 33

FIG. 1. Poorly and irregularly stained cortical cells with postmortem dissolution of the cytoplasm. Child of 5 weeks with diarrhea and bronchopneumonia. Autopsy time 12 hours. Alcohol fixation. Case in Group 1, Division A.

FIG. 2. Irregularly and poorly stained cortical cells with postmortem dissolution of the cytoplasm. Child of 2½ years, dying from kidney tumor and bronchopneumonia. Formalin fixation. Autopsy time 12 hours. Case in Group 1, Division C.

FIG. 3. Microglia and macroglia showing increase in size and number in lower cortical layer. Child of 6 months, dying in convulsions 1 hour after second blood transfusion. Autopsy time 15 hours. Case 9, Group 1, Division C.



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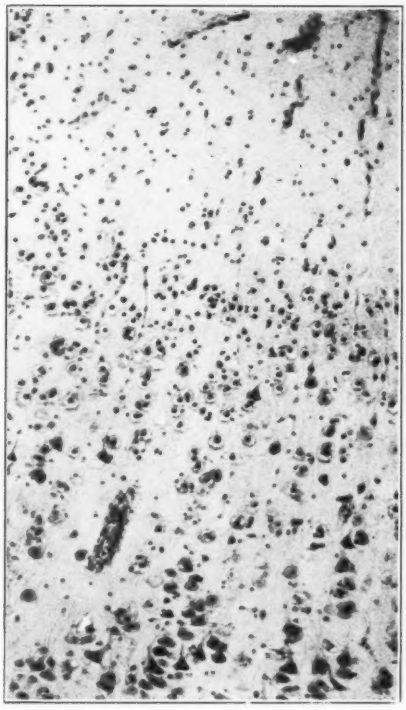

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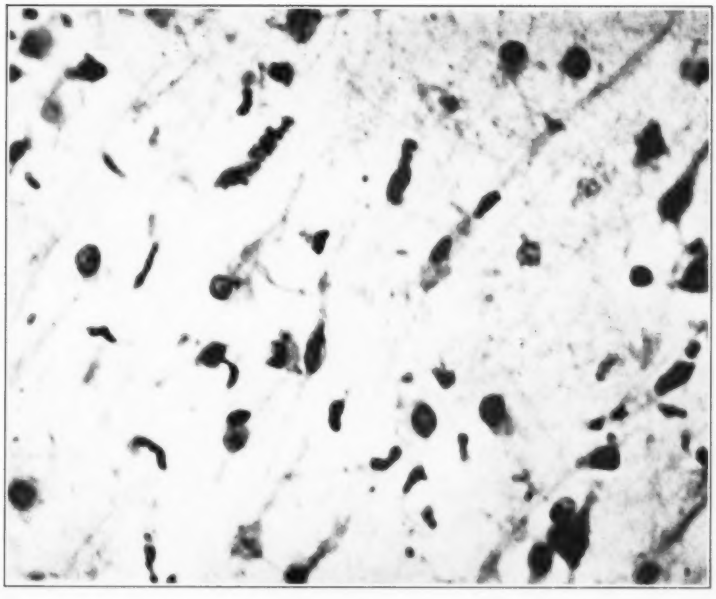

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PLATE 34

FIG. 4. Bleaching of cornu ammonis and temporal cortex in child of 11 months. Death from whooping cough and convulsions. Alcohol fixation. Post-mortem time 7 hours. Case 5, Group 2, Division B.

FIG. 5. Ischemic ganglion cells in Sommer sector of cornu ammonis. Child of 8 months, dying after 2 days of rash and convulsions. Alcohol fixation. Case 7, Group 2, Division B.

FIG. 6. Case of lead poisoning of 5 months duration in child of 2 years. Cortical ganglion cells with distinctly stained cytoplasm and nuclei; also, some loss of ganglion cells and enlarged pale macroglia. Short fixation in formalin. Autopsy time 3 hours. Group 3.

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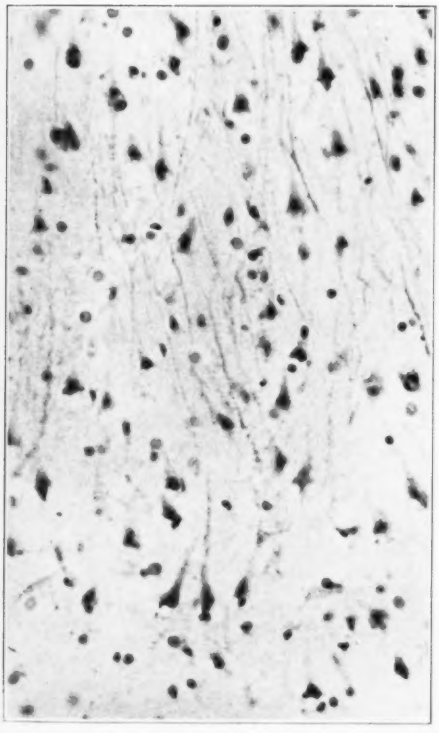

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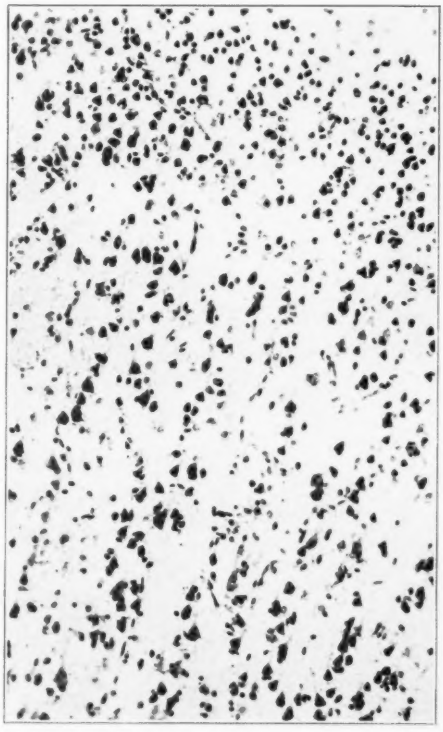

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Tuthill

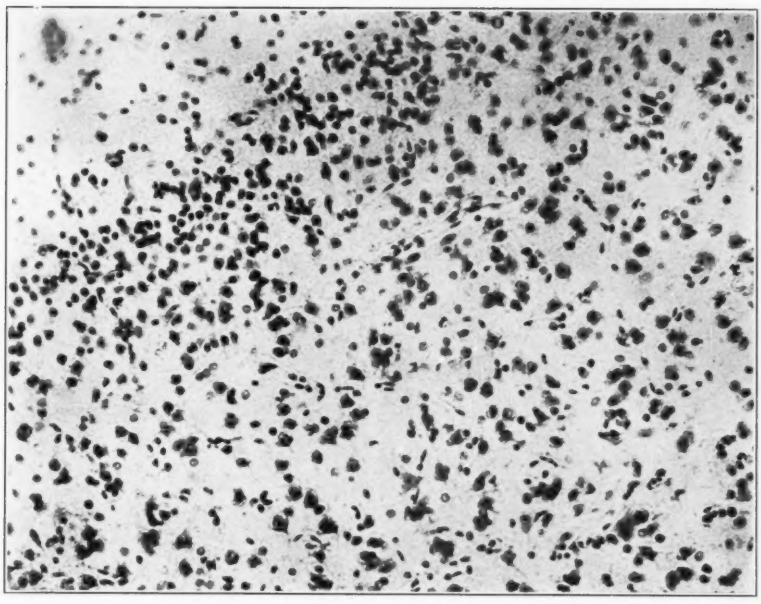
Brains of Infants and Children

PLATE 35

FIG. 7. Well stained cortical cells in case of whooping cough with convulsions and terminal fever of 109° F. Age of child 7 months. Alcohol fixation. Autopsy time, 4 hours. Group 3.

FIG. 8. Three months old normal kitten, killed with ether and autopsied after 12 hours in icebox. Postmortem autolysis of the cytoplasm of the ganglion cells; pale and hyperchromatic staining; enlarged space about the blood vessel and tinting of the nerve tissue. Alcohol fixation.

FIG. 9. Oligodendroglia within cytoplasmic autolyzed cortical ganglion cells. Three months old normal kitten killed with ether. Autopsy after lying dead for 12 hours in cage. Alcohol fixation.



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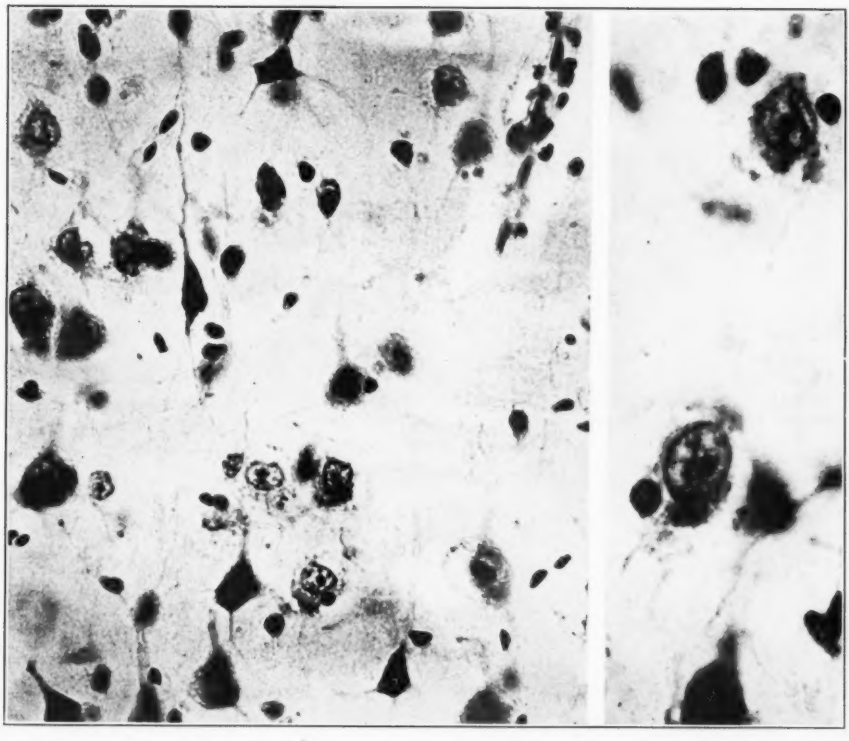

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